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Reproductive ecology variation and molecular phylogeography in the garter snakes of northern New England: (Thamnophis sirtalis sirtalis L 1758 and Thamnophis sirtalis pallidulus Allen 1899)

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REPRODUCTIVE ECOLOGY VARIATION AND MOLECULAR PHYLOGEOGRAPHY IN THE GARTER SNAKES OF NORTHERN NEW ENGLAND:

(*THAMNOPHIS SIRTALIS SIRTALIS L.* 1758, AND *T. S. PALLIDULUS* ALLEN 1899)

BY

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THESIS

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TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

ABSTRACT

REPRODUCTIVE ECOLOGY VARIATION AND MOLECULAR PHYLOGEOGRAPHY IN THE GARTER SNAKES OF NORTHERN NEW ENGLAND:

(*THAMNOPHIS SIRTALIS SIRTALIS L.* 1758, AND *T. S. PALLIDULUS* ALLEN 1899)

By William Stephen Kean

University of New Hampshire, May, 2007

Thamnophis sirtalis, the Common garter snake, is a wide-ranging North American snake exhibiting great variation in morphology and life-history attributes across its range. This thesis studied reproductive variation and molecular phylogeography of *T. s. sirtalis,* the Eastern garter snake, and *T. s. pallidulus,* the Maritime garter snake, in populations from New England in 2005 and 2006. Pregnant female snakes were captured and held in captivity until parturition, and measurements were taken on parent and offspring for comparisons across years and locations. Tissue samples were taken from adult snakes for mtDNA sequencing and subsequent phylogenetic comparisons. No substantial trends were observed in female or clutch characteristics, but offspring were consistently largest from the northernmost population. Low genetic divergence was observed among New England populations, suggesting a recent and continuous recolonization post-glaciation. Both reproductive and molecular data suggest that no substantial differences exist between the two *T. sirtalis* subspecies in New England.

CHAPTER I

REPRODUCTIVE ECOLOGY VARIATION IN NEW ENGLAND *THAMNOPHIS SIRTALIS* POPULATIONS

Introduction

Biology of *Thamnophis sirtalis*

The Common garter snake, *Thamnophis sirtalis,* is a wide-ranging serpent in the family Colubridae, sub-family Natricinea. *T. sirtalis* has the largest range of any snake species in North America, and is found further north on the continent than any other species of snake (Larsen and Gregory, 1989; Ernst and Ernst, 2003). This species is considered a habitat generalist as it is found in many habitats across this range: swamps, river banks, mountainous regions up to elevations of 2500 m, wet meadows, old fields, prairies, deciduous and coniferous forests, and also intertidal areas (Carpenter, 1952; Conant and Collins, 1991; Gregory and Nelson, 1992; Rossman et al., 1996). Furthermore, *T. sirtalis* is a generalist in prey consumed, as it will eat a wide variety of food items: small mammals, birds, eggs, reptiles, amphibians, fish, arthropods, and annelids (Lagler and Salyer II, 1945; Carpenter, 1952; Ernst and Ernst, 2003). Twelve different subspecies of this snake have been described; most of these descriptions are based on regional differences in striping and color morphologies. All snakes within the species have two lateral stripes existing on scale rows two and three, and they may have a central vertebral stripe (Fitch, 1980). Melanistic phenotypes (without stripes) occur in localized populations (King, 1988). Two of the described subspecies occur in the

northeastern United States: *Thamnophis sirtalis sirtalis L.* 1758, and *T. s. pallidulus* Allen 1899 (for more detail on taxonomic history see Chapter 2). Adult snakes tend to be under 90 cm, however the largest specimen observed to date was 137.2 cm (Rossman et al., 1996). Individual snakes may live up to ten years but age-specific survivorship is variable (Fitch, 1999). One wild-caught snake lived 14 years in captivity (Snider and Bowler, 1992).

Thamnophis sirtalis remains one of the best-studied snakes world-wide, however intense studies of this species from the northeastern United States are few. This chapter focuses on the reproductive ecology of *Thamnophis sirtalis* from the New England region of the United States.

Thermoregulation and Activity

Thamnophis sirtalis is an ectothermic species; therefore temperature regulation and related behaviors are important components of life history. Snakes generally bask in sunny or warm locations to acquire heat, and then leave these locations after required heat is gained. Observed voluntary body temperatures varied from 9 to 35 °C in Midwestern popuations (Costanzo et al., 1990). Higher body temperatures are particularly important for digestion of prey items, and digestion temperatures of 24 to 34 °C are frequently preferred (Kitchell, 1969). Higher body temperatures (16 to 26 °C) are preferred during periods of skin-shedding (Kitchell, 1969). Gestating adult females also spend much time basking to gain heat energy for developing young (Gibson and Falls, 1979).

Due to the high latitudes and higher elevations at which *Thamnophis sirtalis* can exist, it must hibernate during the colder times of year. Hibernation occurs in varying

substrates: rock crevices, gravel and dirt embankments, old wells, crayfish burrows, beaver lodges, and any other loose ground substrate that would permit burrowing and body temperature to remain above freezing (Costanzo, 1986; Costanzo, 1989; Larsen and Gregory, 1989). During hibernation, *T. sirtalis* body temperatures generally range from 2 to 7 °C (Carpenter, 1952). Snakes frequently hibernate in large groups in these hibernacula to help prevent freezing, although *T. sirtalis* snakes have been experimentally frozen and survived reanimation (Churchill and Storey, 1992). Studies investigating freeze tolerance in *T. sirtalis,* have found responses of increased free amino acids in blood and increased blood glucose to prevent freezing (Churchill and Storey, 1992).

Activity of *Thamnophis sirtalis* is diurnal during most of the year. *T. sirtalis* was most active in summer months in Ohio from 1300 to 1500 hours (Dalrymple and Reichenbach, 1981), however snakes will also leave night hiding places in the early morning, when prey items such as amphibians may be active. During summer months in warmer regions, snakes will spend earlier parts of the day basking for warmth, and then may go into hiding in the hotter parts of the day to avoid over-heating. Snakes may then repeat the basking in the later evening hours 1800 to 2000 hours to again gain heat (Dalrymple et al., 1991).

Reproductive Features of *Thamnophis sirtalis*

Thamnophis sirtalis is a viviparous species; therefore developing embryos are nourished through a placenta and offspring fully develop within their mother prior to birth. Fertilization is internal, as in all serpents. The species is sexually dimorphic, as males are a maximum 83% of female snout-vent length, and male weight is a maximum 55% of females (Fitch, 1980). Sexual maturity is generally approached between one and

two years of age in males, as they approach a snout-vent length of 36 to 39 cm (Rossman et al., 1996) . Sexual maturity in females is reached between years two and three at snout vent-lengths of 42 to 55 cm (Rossman et al., 1996). In a northeastern population found off the coast of Nova Scotia, some females were found to be reproductive at smaller sizes (< 39 cm) (Barnes et al., 2006). *T. sirtalis* is also considered a capital-breeding species, which means energy for a given set of offspring is sequestered prior to the reproductive event instead of gathered during parturition.

Costs of Reproduction in Viviparous Reptiles

Energy acquisition and usage is a key determinant to an animal's life history and fitness. *Thamnophis* snakes are viviparous, capital-breeding species (Ford and Seigel, 1989). This combination of reproductive strategies creates a unique set of dangers and stresses for the reproducing individual; energy must be spent to develop young internally, but most of the energy comes from previous stores (Shine, 1980; Bonnet et al., 1998; Bonnet et al., 2002). Moreover, gestation periods are long and can be energetically expensive for a pregnant female as commitments often need to be made between basking for heat energy, and foraging for current sustenance. Capital-breeding, viviparous snakes have often been observed to undergo a period of "facultative anorexia" during gestation, where females essentially ingest no prey items during pregnancy (Lourdais et al., 2002a). Survival of females during parturition is also a potential cost of reproduction, as gravid females are less likely to escape capture by predators- particularly towards the end of gestation (Seigel et al., 1987; Bonnet and Naulleau, 1996).

A trade-off must be found between supplying maximum reproductive effort for a given year (i.e. maximum clutch mass in offspring size and number), and reserving

energy stores for existence beyond the reproductive event and for future reproductive events. As energy spent per individual offspring increases, the number of offspring a parent can produce must also become limited, thus creating a trade-off in individual fitness, offspring number, and offspring size (Smith and Fretwell, 1974). In this case, as energy allocated per individual offspring may be increased, the parent's overall fitness is increased (Smith and Fretwell, 1974). Investing optimally in reproduction is a difficult task for a long-lived organism, especially in an unstable, fluctuating environment (Schaffer, 1974). Many capital-breeding snakes, such as *T. sirtalis*, rarely reproduce in consecutive years as seasons between reproductive events must be used to regain energy stores.

Trends in Reproductive Investment and Clutch Characteristics

Reproductive energy limitations and differences in reproductive strategies lead to commitments which must be made if optimal reproduction is to be obtained. For a pregnant female, is it closer to optimal to have fewer but more offspring, or a larger number of smaller offspring?

Seigel et al. (1985) found that within four species of North American snakes clutch size varied greatly among populations within years even after making corrections for female body size. Larger clutches of an oviparous species (*Diadophis punctatus*) were well-correlated with higher than normal rainfall, and higher autumn rainfall. These researchers found that "good" or "bad" years could exist for reproductive traits, and thus cautioned future investigators against using one season's worth of data for comparative studies between geographic populations.

Relative clutch mass (RCM) is an often-used index of reproductive effort; it is based upon clutch mass and postpartum female weight. It creates a method to look at investment in clutch mass per unit of adult female body mass. Seigel et al. (1986) studied patterns of relative clutch mass within sixteen species of viviparous colubrids, and seven species of viviparous viperids. Colubrid snakes yielded significantly lower relative clutch masses for longer females (based on snout-vent length), than smaller snakes. Viperids showed a similar trend, but the result was not statistically significant. In oviparous colubrids, Seigel et al. found a positive, yet statistically insignificant relationship with larger female snout-vent length and relative clutch mass. These authors suggested that these relationships may be due to the larger costs of viviparity compared with oviparity (Seigel et al., 1986). Moreover, across at all colubrid species studied, smaller species were able to bear greater RCM perhaps because of greater longevity and habitat utilization. Smaller snakes are more cryptic and better able to hide from predators than larger, heavier species that may spend more time basking in open spaces. Bonnet and Naulleau (1996), found that larger and pregnant snakes *Vipera aspis* escaped capture attempts less frequently than smaller and non-reproductive conspecifics.

In the viviparous brown snake, *Storeria dekayi,* female size and condition at ovulation were linked to higher offspring number (King, 1993b). In addition, larger females and females in good condition produced smaller offspring, and offspring in poorer condition. These results were attributed to females assuming they would have more chances to reproduce, so not as many large, healthy offspring were produced. This study also showed longer captivity produced offspring in poorer condition (King, 1993b). In the redbellied snake, *Storeria occipitomaculata,* a two-year study reported that litter

(clutch) mass, and litter size correlated positively with female size, yet mean offspring size showed no direct correlation to female size (Brodie III and Ducey, 1989). These findings suggested that reproductive redbellied snakes invested more in greater clutch sizes rather than larger offspring.

In the checkered garter snake, *Thamnophis marcianus*, diets of gravid females were manipulated to investigate how differential feeding regimes affected offspring characteristics. Immediately following hibernation, researchers placed snakes on either a high energy or low energy feeding scheme of bullfrog (*Rana catesbeiana*) tadpoles (30% of body weight/ week, or 10% of body weight/ week). Clutch size and clutch mass were significantly higher in high energy females, than in low energy females. On the contrary, relative clutch mass, and offspring size were not affected by feeding regime (Ford and Seigel, 1989). This study suggested that when comparing characteristics intraspecifically, one should be sure to use traits which are phenotypically stable.

Differential feeding regimes and offspring traits were also tested by Gregory and Skebo (1998), who partitioned two small (n = 9) groups of pregnant *Thamnophis elegans* into those which were given food, and those not given food during captivity. This study revealed no significant difference in offspring mass, mean offspring snout-vent length, litter mass, or litter size between groups in the different feeding regimes. However, post-partum condition of adult females was different between the two groups. Despite their lack of statistically significant results, this research group questioned if better conditioned females might allocate more to current reproduction versus females in poorer condition investing more for future reproduction, contradicting the findings of King's (1993b)

study with brown snakes. This therefore also points to monitoring reproductive costs in more than one year.

Geographic and Ecological Trends in Clutch and Reproductive Attributes

Other studies have attempted to find evidence for geographic or ecological trends in reproductive attributes. Definitions and manifestations of optimal reproduction and optimal body size must be different in different habitats with differing conditions. Such condition parameters might be temperature, precipitation, and prey availability. Experiments to find evidence of geographic trends in reproductive attributes have often been inconclusive or contradictory. Table 1.1 shows clutch statistics for *Thamnophis sirtalis* reproductive studies conducted in locations across North America. Great differences are seen among locations and years.

Table 1.1: *Thamnophis sirtalis* clutch sizes.

Location	Mean Clutch Size	# sampled (N)	Source
Manitoba	18.8	30	(Gregory and Larsen, 1993)
Maryland	32.5	11	(McCauley, 1945)
Michigan	16.9	8	(Burt, 1928)
Michigan	18	20	(Carpenter, 1952)
New Hampshire	12.9	104	(Zehr, 1962)
Alberta	12.5	23	(Larsen et al., 1993)
California	17	6	(Cover and Boyer, 1988)

Due to the great range of *Thamnophis sirtalis* in its many subspecies, the species stands as a model organism to investigate such hypotheses regarding trade-offs and geographic trends. Several researchers have hypothesized that such trends would be most apparent when studying populations at the extreme limits of the ranges - particularly when comparing differences between northern and southern populations. Inconclusive and contradictory results have been found, particularly when compared on different scales.

Gregory and Larsen (1993), looked at litter characteristics from multiple sites across Canadian populations and observed a few trends. When comparing litter characteristics from snakes captured near the northern limit of the range in Wood Buffalo National Park at 59° 49' N (Northwest Territories, Canada) to litters taken further south in Manitoba, they observed that females further north approached sexual maturity at larger sizes (Larsen et al., 1993). Neonates were larger and clutches were smaller at the northern site. Despite this finding, when looking at populations across Canada, no substantial north-south trends were found (Gregory and Larsen, 1993). They did seem to find evidence of an east-west cline, as snakes in western Canada reached sexual maturity at larger body sizes than snakes in eastern Canadian populations and neonates were larger at time of birth also in western populations (Gregory, 1996). In addition, litter sizes were smaller for similar-sized snakes in the western populations compared to eastern populations. When subspecies designations were employed in calculations, more substantial differences were observed among populations of the same subspecies, than among subspecies. Limitations to this larger review were that data were taken from different years, and consistencies in counting methods were suspect. Moreover, data points from eastern North America were particularly lacking.

Research Questions and Hypotheses

The primary goal of this study was to test for geographic trends in female morphometric traits, offspring morphometric traits, and clutch characteristics among populations of *Thamnophis sirtalis* snakes in New England. In the process, I summarized clutch characteristics and morphometric measurements for four separate populations of *Thamnophis sirtalis* snakes in two consecutive years. These populations existed at

different latitudes ranging from northern New Hampshire through south-central Massachussetts. I aimed to further infer if differences existed in clutch characteristics or morphometric measurements between the current subspecies classifications of *T. s. sirtalis,* and *T. s. pallidulus*. I also investigated if female garter snakes reproduce in successive years, as most previous literature suggests that in northern populations, they do not.

Therefore my null hypotheses were:

I. H_0 : There are no geographic patterns in mean clutch size, mean adult snout-length, mean female prepartum weight, mean female postpartum weight, mean clutch mass, mean relative clutch mass, mean offspring snout-vent length and mean offspring weight.

H_A : There are geographic trends in the stated parameters.

II. H_0 : There are no differences in mean clutch size, mean adult snout-length, mean female prepartum weight, mean female postpartum weight, mean clutch mass, mean relative clutch mass, mean offspring snout-vent length and mean offspring weight between the two subspecies of *Thamnophis sirtalis* snakes found in New England *T. s. sirtalis* (the Eastern garter snake) and *T. s. pallidulus* (the Maritime garter snake).

H_A : There are differences in the stated parameters between the two subspecies

Based on optimal reproduction theory, and previous results (Larsen et al., 1993), I predicted that snakes captured further north would yield higher clutch masses. This could come from either larger clutch sizes in one reproductive event, or overall larger offspring at time of birth. Moreover, I predicted that pregnant females would be heavier for their length, at the time of reproduction and post reproduction, than snakes found further south.

Due to fewer days of high temperatures in the northern regions of New England, I hypothesize that female snakes need to create larger stores of energy prior to the reproductive event, in order to give offspring and themselves more energy post-parturition.

Testing differences in subspecies reproductive outputs and morphometrics, I predicted that *Thamnophis s. pallidulus* will have larger females and offspring than *T. s. sirtalis*. Some prior observations have suggested *T. s. pallidulus* tends to be "girthy" (Tennant, 2003).

I also predicted that I would not observe female adult snakes reproducing in consecutive years.

Methods

Sampling Locations

The study locations used in this experiment were: Westborough, Massachusetts, at the Massachusetts Fish and Wildlife Headquarters; Durham, New Hampshire, at the University of New Hampshire's East and West Foss Farm Properties; in Center Ossipee, NH, at the University of New Hampshire's Lovell River Property, and in Pittsburg, NH, at the Connecticut Lakes Natural Area. Descriptions of these locations can be found in Table 1.2 and the location map Figure 1.1. These sites were selected to create a latitudinal transect which crossed the subspecies boundaries between *T. s. sirtalis,* and *T. s. pallidulus* (Tennant, 2003). This transect was also designed so populations in different environments could be compared using latitude as a proxy for different habitat conditions.

Table 1.2: Study site descriptions.

Site	Coordinates	Habitat Description
Westborough, MA (Fish and Wildlife Headquarters)	71°37'38.04"W 42°17'41.72"N	Old field community. Mixed deciduous trees of maple (*Acer sp.)*, oak (*Fagus),* hickory (*Carya*) stands in between early successional fields of grass species (*Poaceae)*, prairie flowers such as goldenrod (*Solidago sp.)*, sumac (*Rhus)*, and raspberry *Rubus* present.
Durham, NH (East and West Foss Farm Properties, University of New Hampshire)	70°56'42.58"W 43°07'20.21"N	Wet meadow, and early successional prairie. Within the wet meadow reed-canary grass (*Phalaris arundinacea),* sedges (*Carex sp.*) and cat-tail (*Typha sp.)* are dominant. Raspberry (*Rubus*), sumac (*Rhus*), grass species (*Poaceae*), and honeysuckle (*Lonicera*) are prevalent in the early successional region.
Ossipee, NH (Lovell River Property ,University of New Hampshire)	71°09'59.14"W 43°46'34.88"N	Pine barrens community. Dominant tree species are red, white and pitch pine (*Pinus sp.).* Ferns (*Osmunda*) and raspberry (*Rubus)* were present in the patch where snakes were collected.
Pittsburg, NH (Connecticut Lakes Natural Area)	71°09'58.38"W 45°11'45.45"N	Tree canopy is dominated by spruce (*Picea)* and fir (*Abies)* species. Understory species contain grasses (*Poaceae*), sedges (*Cyperaceae*) , and alder (*Alnus).* This location has many beaver ponds nearby.

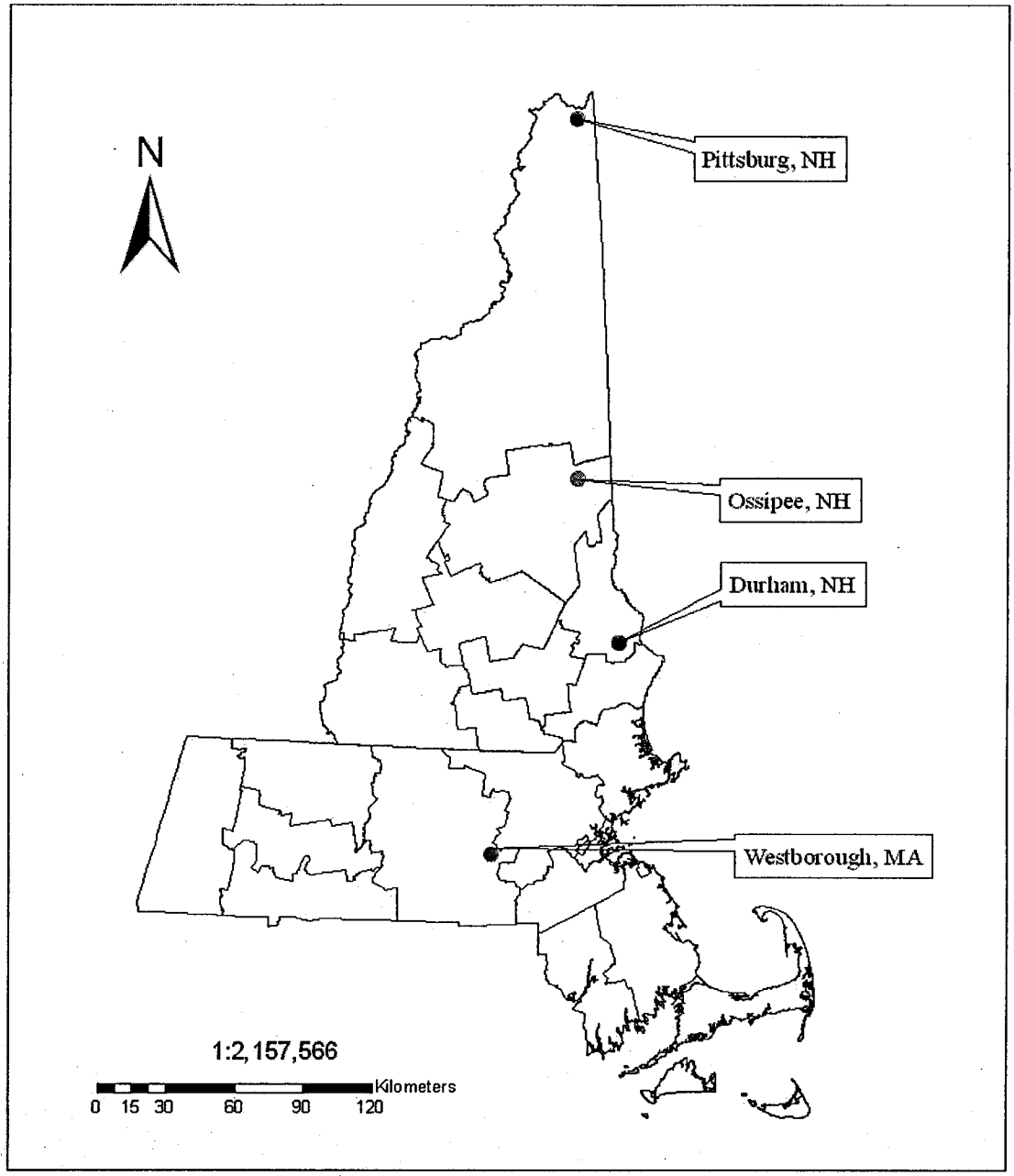


Figure 1.1: Map of *Thamnophis sirtalis* collection sites in New England.

Collection Procedures

Sampling plots were established by July 1 in 2005, with cover objects laid out at all locations. Cover objects consisted of 4 or 6 mil black tarps cut to dimensions approximately 1.5 by 2 meters in size, doubled over, and then attached to the ground with garden staples in each corner. Using cover objects to attract garter snakes is a common method for capture, particularly for pregnant females, as the dark substance heats up and creates a warmer microenvironment. Approximate numbers of tarps at each location were as follows in 2005: Westborough, Mass: 28; Durham New Hampshire: 20, Center Ossipee, NH: 15; and Pittsburg, NH: 20.

Snake collection was conducted most often between 1500 and 1800 hours. Tarps were temporarily lifted to see if snakes were present. Snakes were then hand captured, and placed in nylon-cloth bags for transport back to the lab at the University of New Hampshire. I also searched around vegetation, logs, rocks, and other debris at these study sites.

In 2005, snake collection at the Westborough, MA, and Durham, NH study sites ran primarily from June 3 through July 15. Attempts at snake collection in the other two northern sites ran from June 15 through August 1.

Numbers of sampling tarps in 2006 at each location were: Westborough, Mass: 48; Durham, NH: 30; Center Ossipee, NH: 6, Pittsburg, NH 18. The sampling time periods in 2006 were extended longer for the Westborough, MA, Durham, NH and Ossipee, NH (May 25 through August 1), and shortened in the northern location due to permit restrictions (June 25 through August 4 in Pittsburg, NH). All snakes collected at the Pittsburg, NH site were collected by visual spot and hand collection, despite cover

objects being employed, as cover objects seemed ineffective at this location (W. Kean personal observation)

Laboratory Procedures

Within 48 hours of capture, all snakes were weighed, marked with a paint marker, and placed into a group enclosure with other snakes from the same location. The group enclosures varied in size and shape from 12 liter plastic sweater boxes to 260 liter (65 gallon) glass fish tanks. It was assumed that effects of group living or size of enclosure were insignificant, as snakes were frequently found during collection under the same tarp (W. Kean, personal observation), and in wild populations females den together (Larsen and Gregory, 1989). No more than two adult snakes were kept in the smaller containers, whereas up to ten or twelve snakes were contained for the gestation period in the larger containers. All enclosures had tight fitting lids fastened with weights or binder clips to prevent snake escape.

Snakes enclosures were lined with newspaper and pieces of cardboard were provided as cover objects within the containers. Water was offered ad *libitum*. Snakes were fed bait worms (Canadian night crawlers: *Lubricum terrestris*, or European night crawlers *Eisenia hortensis*) approximately every four to five days during gestation from time of capture through parturition. Frequently, snakes would not eat within ten days of parturition (W. Kean personal observation). These observations are consistent with other field observations (Rossman et al., 1996). Cages were cleaned at least once a week, and at this time, snakes were weighed to track weight gain and health of animals. Reptiles frequently show weight loss if they are ill (Rossman et al., 1996). In 2005, the ambient laboratory temperature was maintained between 24°C and 26.5°C. In 2006, 100 watt

incandescent light bulbs were placed near terrariums to create a temperature gradient within snake enclosures. This temperature gradient typically existed between 23°C and 28°C. This higher temperature maximum was thought to be more like daily high temperatures found in study locations.

Once parturition was observed in one snake from a given sampling location, all snakes from that given location were separated individually from larger group enclosures into 12 or 20 liter containers, in order to avoid confusion with future litter counts. Once parturition occurred from a given snake, all offspring within the litter were counted to get a litter size count, weighed on a Mettler 440 Deltarange® balance to the nearest 0.01 gram, and snout-vent lengths were measured to the nearest 0.1 mm. All still-born offspring were weighed, measured, and counted. Depending on the condition of the still born or degree of deformity, some of these data were not available for measure. After litter measurements were taken, adult female measurements of weight (to the nearest 0.1 g) and snout-vent length (to the nearest 0.1 cm) were also recorded.

Within ten days of parturition adult and offspring snakes were returned to the original site of capture. In 2005, all adult female snakes were injected with a PIT tag, in order to collect recapture success data in 2006.

Data Analysis

Data analysis was conducted in JMP 6.02 (SAS Institute Inc.). Two separate variables, clutch mass and relative clutch mass (RCM), were calculated as follows:

clutch mass = Σ offspring weights (g)/ female

relative clutch mass = clutch mass/ female prepartum weight

Natural logarithm (LN) transformations were conducted on all morphometric data to fit assumptions about normal distribution to prepare for regression analyses and analysis of covariance (ANCOVA). Such transformations have also been made in other similar studies (Dunlap and Lang, 1990; Larsen et al., 1993; King, 1993b). Comparisons of differences in means were completed for the following measured variables: relative clutch mass, clutch mass, clutch size, female snout vent length (SVL), female pre-and post-partum weight, offspring SVL and offspring weight. Analyses involving offspring were averaged across the whole clutch to meet assumptions about pseudoreplication. Furthermore, tests with offspring data were conducted using two different data-sets: live plus still-borne offspring, and only live offspring. For these analyses location and year were first crossed as the independent variable to see if interactions existed between these two variables. Where significant differences were found in means ($P < 0.05$), Tukey-Kramer post-hoc tests were conducted to see which populations or years differed. If significant interactions were observed between the variables, pair-wise tests comparing slopes were also conducted to find where the significant interaction existed.

Days in captivity were also counted to look for influences of time in lab for certain parameters. As suggested by previous researchers, effects in the lab could influence overall results. Days in captivity was regressed against the parameters of female-post partum mass, relative clutch mass, offspring snout-vent length and offspring weight.

Clutch mass and sesiduals of female prepartum weight and snout-vent length were also regressed to investigate if body condition of pregnant females prior to captivity influenced resulting clutch masses.

Results

Collection and Recapture Results

Between the two years sampled, 103 clutches were counted among the four populations (Table 1.3).

Table 1.3 : 2005 and 2006 *Thamnophis sirtalis* clutch counts by location

Location	2005	2006	Total
Westborough, MA	11	33	44
Durham, NH	9	20	29
Ossipee, NH	3	5	8
Pittsburg, NH	1	21	22

Of the 24 reproducing snakes which were PIT-tagged and released after parturition in 2005, four were recaptured, and none were reproductive in 2006 (Table 1.4).

Table 1.4 : PIT-Tag Recapture data by location

Location	Total # PIT-Tagged in 2005	Reproduced # PIT-Tagged in 2005	Total # Recaptured in 2006	# Recaptured and Reproduced in 2005	# Recaptured and Reproduced in 2006	# Recaptured and Reproduced in both 2005 and 2006
Westborough, MA	10	10	2 (20%)	2 (20%)	0 (0%)	0 (0%)
Durham, NH	13	9	3 (23%)	2 (22%)	0 (0%)	0 (0%)
Ossipee, NH	6	3	1 (17%)	0 (0%)	0 (0%)	0 (0%)
Pittsburg, NH	1	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Tests of Differences Between Years and Locations in Female Characteristics

Table 1.5 shows results for analysis of covariance (ANCOVA) tests testing for differences in female parameters measured across the two years and four populations. No significant relationship was noticed between years and female snout-vent length (DF = 1, F = 0.1363, P = 0.7128) however a significant relationship was observed between location and female snout-vent length (DF = 3, F = 5.1982, P = 0.0023) (Table 1.5, Figure 1.2). According to Tukey-Kramer pair-wise comparisons tests, adult females captured from Westborough, MA, and Pittsburg, NH were significantly longer than snakes from Durham, NH.

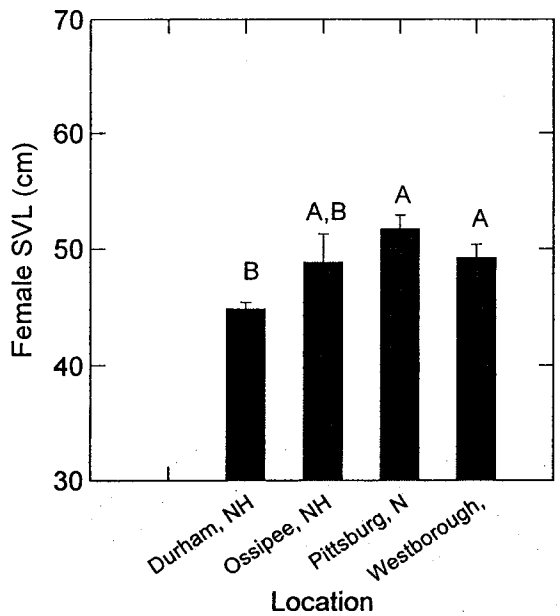


Figure 1.2 Mean female snout vent length (cm) versus location (g). This figure shows differences in mean female snout-vent length measurements taken on pregnant *Thamnophis sirtalis* garter snakes from four different populations in New England. Absolute mean values are shown, however tests for differences were calculated based on a natural logarithm transformation of the snout-vent length values.

Table 1.5 : ANCOVA Results for female morphometric and clutch measurements.

Variable										Figure
LN Female snout-vent length (cm)					Effect Tests					
	Source	DF	Sum of Squares	Mean Square	F Ratio	Source	DF	Sum of Squares	F Ratio	**Prob > F**	
	Model	7	0.31724	0.04532	3.4179	Location	3	0.20678	5.1982	**0.0023**	1.2
	Error	93	1.23313	0.01326	**Prob > F**	Year	1	0.00181	0.1363	**0.7128**	
	C. Total	100	1.55037		**0.0027**	Location*Year	3	0.05575	1.4015	**0.2473**	
LN Female prepartum weight (g)											
	Model	7	2.42891	0.34699	3.0457	Location	3	1.99259	5.83	**0.0011**	1.3
	Error	93	10.5952	0.11393	**Prob > F**	Year	1	0.07287	0.6396	**0.4259**	
	C. Total	100	13.0241		**0.0063**	Location*Year	3	0.33795	0.9888	**0.4017**	
LN Female postpartum weight (g)											
	Model	7	2.94613	0.42088	3.6374	Location	3	1.67555	4.827	**0.0037**	1.4
	Error	90	10.4136	0.11571	**Prob > F**	Year	1	0.26586	2.2977	**0.1331**	
	C. Total	97	13.3598		**0.0017**	Location*Year	3	0.41545	1.1968	**0.3156**	
LN Clutch mass (g)											
	Model	7	3.06938	0.43848	2.2382	Location	3	2.1796	3.7085	**0.0144**	1.5
	Error	90	17.6318	0.19591	**Prob > F**	Year	1	0.0494	0.2522	**0.6168**	
	C. Total	97	20.7012		**0.0382**	Location*Year	3	0.49522	0.8426	**0.4741**	
Clutch size											
	Model	7	505.092	72.156	2.0455	Location	3	412.44	3.8973	**0.0113**	1.6
	Error	94	3315.93	35.2758	**Prob > F**	Year	1	3.52699	0.1	**0.7526**	
	C. Total	101	3821.02		**0.0573**	Location*Year	3	154.768	1.4625	**0.2298**	
Relative clutch mass											
	Model	7	0.29415	0.04202	2.1784	Location	3	0.09249	1.5983	**0.1954**	1.7
	Error	89	1.71676	0.01929	**Prob > F**	Year	1	0.00636	0.3299	**0.5672**	
	C. Total	96	2.0109		**0.0435**	Location*Year	3	0.12703	2.1952	**0.0942**	

Female prepartum and postpartum weights showed no significant differences between years, however significant differences were observed among locations in pre- and postpartum weights (DF = 3, F = 5.83, P = 0.0011; DF = 3, F = 4.827, $P = 0.0037$) (Table 1.5, Figure 1.3, 1.4). Prepartum weights were significantly higher in Westborough, MA, and Pittsburgh, NH when compared to Durham, NH (Figure 1.3). No difference could be detected between Ossipee, NH and any of the other populations. Postpartum masses were significantly different between Pittsburg, NH and Durham, NH, and the other two populations were indistinguishable from either that of Pittsburg, NH or Durham, NH (Figure 1.4).

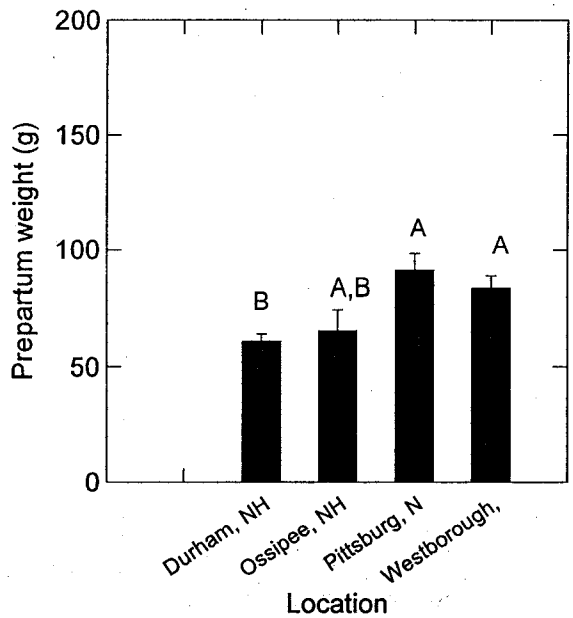


Figure 1.3. Mean prepartum weight (g) versus location. This figure shows differences in mean prepartum weight measurements taken on pregnant *Thamnophis sirtalis* garter snakes from four different populations in New England. Absolute mean values are shown, however tests for differences were calculated based on a natural logarithm transformation of the prepartum weight values.

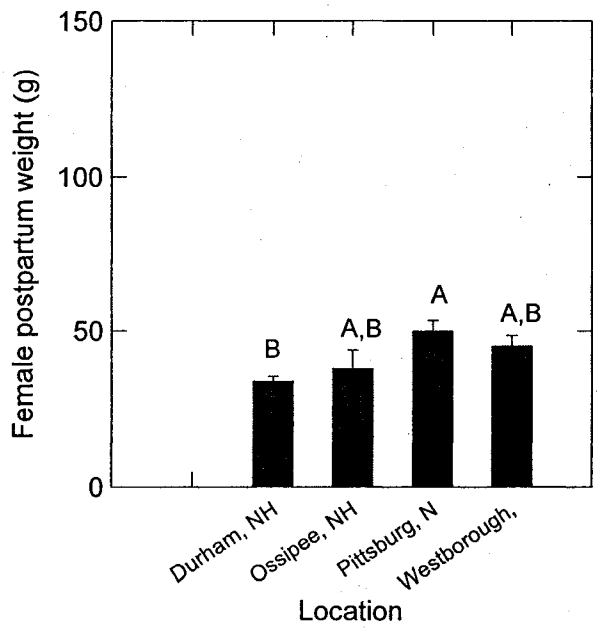


Figure 1.4. Mean postpartum weight (g) versus location. This figure shows differences in mean postpartum weight measurements taken on pregnant *Thamnophis sirtalis* garter snakes from four different populations in New England. Absolute mean values are shown, however tests for differences were calculated based on a natural logarithm transformation of the weight values.

Clutch masses (Table 1.5, Figure 1.5) showed no significant difference between years (DF = 1, F = 0.2522, $P = 0.6168$, but a significant relationship was observed between location and clutch mass (DF = 3, F = 3.7085, $P = 0.0144$). Tukey-Kramer pair-wise tests showed Pittsburg, NH female snakes had significantly larger clutch masses, when compared to Durham, NH snakes. Again, Westborough and Ossipee populations were indistinguishable from the above two populations (Figure 1.5).

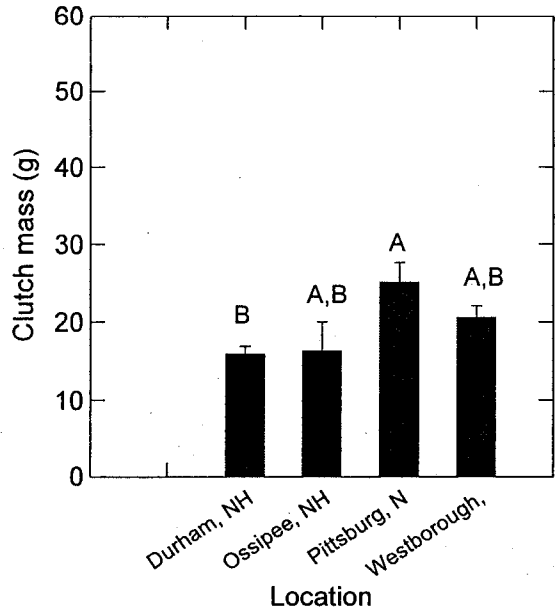


Figure 1.5. Mean clutch mass (g) versus location. This figure shows differences in mean clutch mass values for four populations of *Thamnophis sirtalis* snakes in two consecutive years.

Clutch sizes (Table 1.5, Figure 1.6) showed significant differences among locations (DF = 3, F = 3.8973, $P = 0.0113$), but not between years (DF = 1, F = 0.1, $P = 0.7526$). In pair-wise comparisons tests between locations, significant differences were found between the Westborough population, and the Durham population, suggesting Westborough clutch sizes were larger than those of Durham (Figure 1.6). Snakes from Ossipee or Pittsburg, NH were not significantly different from either Westborough or Durham snakes.

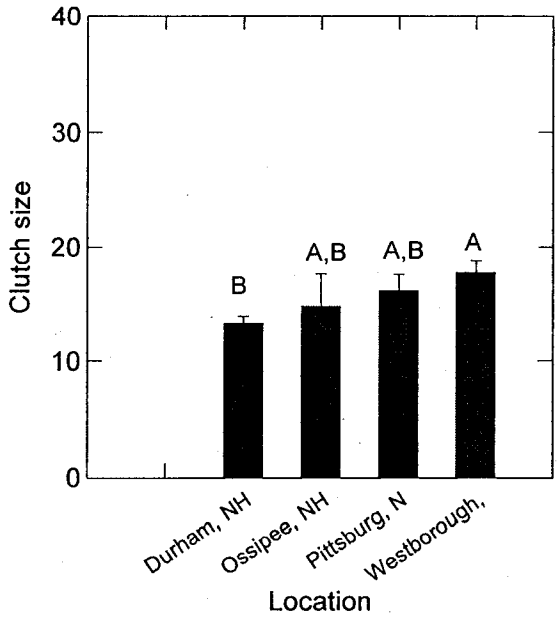


Figure 1.6 Clutch size versus location. This figure shows differences in mean clutch size between four populations of *Thamnophis sirtalis* garter snakes in New England.

The only female parameter which did not show significant differences in either year or location was relative clutch mass (RCM) (Table 1.5, Figure 1.7). All mean relative clutch masses for the populations were within similar ranges (Figure 1.7).

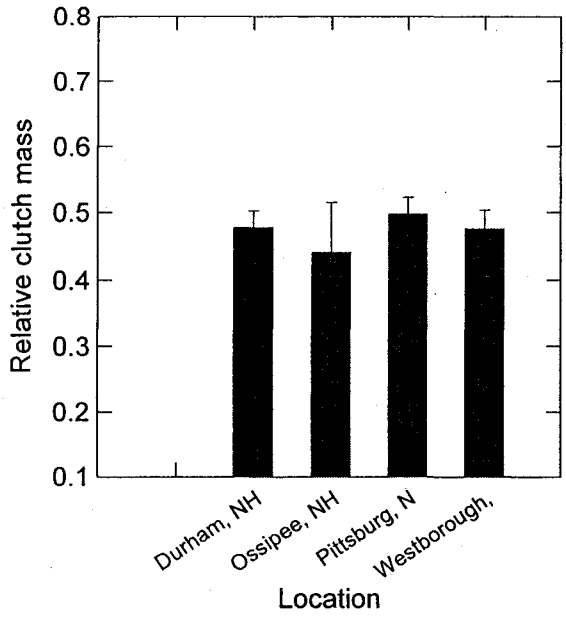


Figure 1.7. Relative clutch mass versus location. This figure charts relative clutch masses for four populations of *Thamnophis sirtalis* garter snakes in New England.

Tests of Differences in Years and Locations of Offspring Snakes

Table 1.6 shows ANCOVA results, testing differences in offspring measurements between years and locations sampled. A significant interaction was observed between years and locations, and pair-wise comparisons tests (Tables 1.7, 1.8) show that only Durham snakes had significant different values between years. Means of snout-vent lengths were longer in 2006 than in 2005. Because only one population differed between years, I continued the analysis as if no interaction was observed. Therefore, in terms of offspring snout-vent length, Pittsburg, NH offspring were significantly longer than all three other populations of snakes, whether all offspring (live and still-born) (Figure 1.8) were used or only live offspring were used in the calculation (Figure 1.9).

Table 1.6. ANCOVA Results for offspring measurements

Variable										Figure	Pair-wise year and location table
LN Mean offspring snout-vent length (L+S)					Effects Tests						
Source	DF	Sum of Squares	Mean Square	F Ratio	Source	DF	Sum of Squares	F Ratio	**Prob > F**		
Model	7	0.24679	0.03526	10.0682	Location	3	0.03437	3.2719	**0.0248**	1.8	1.7
Error	89	0.31164	0.0035	**Prob > F**	Year	1	0.0026	0.7417	**0.3914**		
C. Total	96	0.55843		**<.0001**	Location*Year	3	0.03555	3.3837	**0.0216**		
LN Mean offspring snout-vent length (L)											
Model	7	0.24531	0.03505	10.3611	Location	3	0.03668	3.6149	**0.0164**	1.9	1.8
Error	86	0.29088	0.00338	**Prob > F**	Year	1	0.00184	0.5436	**0.4629**		
C. Total	93	0.53619		**<.0001**	Location*Year	3	0.0294	2.8971	**0.0397**		
LN Mean offspring weight (L+S)											
Model	7	1.32844	0.18978	6.8229	Location	3	0.37163	4.4536	**0.0058**	1.10	
Error	90	2.50332	0.02782	**Prob > F**	Year	1	0.08904	3.2012	**0.0769**		
C. Total	97	3.83176		**<.0001**	Location*Year	3	0.13364	1.6016	**0.1946**		
LN Mean offspring weight (L)											
Model	7	1.3124	0.18749	7.8456	Location	3	0.35645	4.972	**0.0031**	1.11	
Error	86	2.05513	0.0239	**Prob > F**	Year	1	0.03873	1.6209	**0.2064**		
C. Total	93	3.36753		**<.0001**	Location*Year	3	0.07328	1.0221	**0.387**		

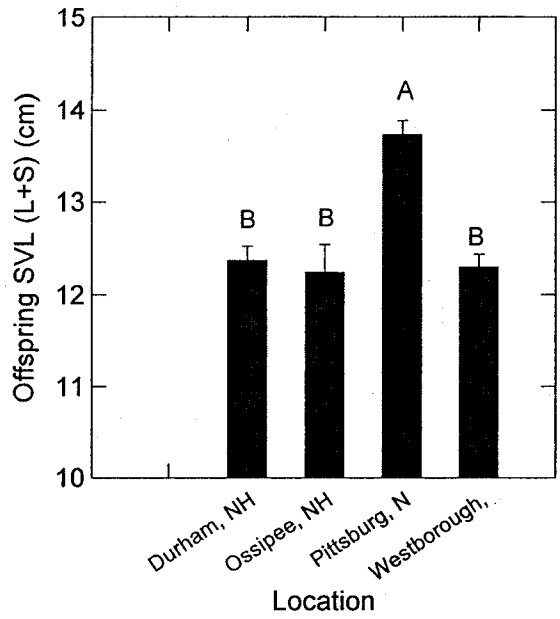


Figure 1.8. Mean offspring snout-vent length (cm) versus location. This figure shows differences in mean snout-vent length of offspring *Thamnophis sirtalis* garter snakes from four populations in New England. Absolute values are represented, however tests for differences were completed using natural logarithm transformed values. The data set used to complete this analysis consisted of all live and still-born offspring counted.

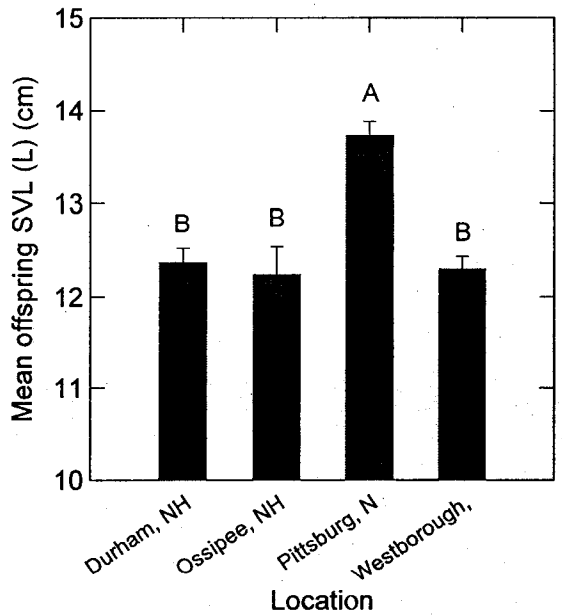


Figure 1.9. Mean offspring snout-vent length (cm) versus location. This figure shows differences in mean snout-vent length of offspring *Thamnophis sirtalis* garter snakes from four populations in New England. Absolute values are represented, however tests for differences were completed using natural logarithm transformed values. The data set used to complete this analysis consisted of only live offspring counted.

Table 1.7 : Pair-wise tests of LN mean offspring snout-vent lengths (cm) (L+S) by location and year

Location	**Analysis of Variance**					
Westborough, MA	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
	Year	1	0.00000563	5.63E-06	0.0014	**0.9706**
	Error	35	0.14293778	0.004084		
	C. Total	36	0.14294341			
Durham, NH	Year	1	0.04231093	0.042311	12.3549	**0.0016**
	Error	26	0.08904029	0.003425		
	C. Total	27	0.13135122			
Ossipee, NH	Year	1	0.00418256	0.004183	1.9688	**0.2195**
	Error	5	0.01062204	0.002124		
	C. Total	6	0.01480459			
Pittsburg, NH	Year	1	0.00141197	0.001412	0.4812	**0.4959**
	Error	20	0.05868762	0.002934		
	C. Total	21	0.06009959			

Table 1.8 : Pair-wise tests of LN mean offspring snout-vent lengths (cm) (L) by location and year

Location	**Analysis of Variance**					
Westborough, MA	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
	Year	1	0.00078802	0.000788	0.183	**0.6715**
	Error	34	0.14637025	0.004305		
	C. Total	35	0.14715827			
Durham, NH	Source	DF	Sum of Squares	Mean Square	F Ratio	**0.0014**
	Year	1	0.03746059	0.037461	12.6958	
	Error	26	0.07671614	0.002951		
	C. Total	27	0.11417672			
Ossipee, NH	Source	DF	Sum of Squares	Mean Square	F Ratio	**0.1816**
	Year	1	0.00574626	0.005746	2.6084	
	Error	4	0.00881181	0.002203		
	C. Total	5	0.01455807			
Pittsburg, NH	Source	DF	Sum of Squares	Mean Square	F Ratio	**0.5340**
	Year	1	0.00108436	0.001084	0.4004	
	Error	20	0.05416193	0.002708		
	C. Total	21	0.05524629			

No differences in offspring weight were found between years with either live or live plus still-born offspring data set, however differences were observed among locations (DF =3, F = 4.4536, $P = 0.0058$; DF = 3, F = 4.972, $P = 0.0031$) (Table 4, Figure 1.10, 1.11). In both cases Pittsburg, NH snakes were significantly heavier at time of birth, when compared to all three other populations of snakes (Figure 1.10, 1.11).

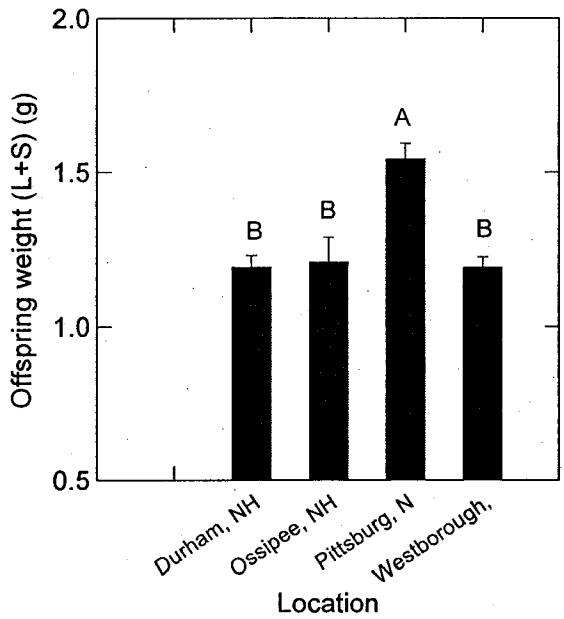


Figure 1.10. Mean offspring weight (g) versus location. This figure shows differences in mean weight of offspring *Thamnophis sirtalis* garter snakes from four populations in New England. Absolute values are represented, however tests for differences were completed using natural logarithm transformed values. The data set used to complete this consisted of all live and still-born offspring counted.

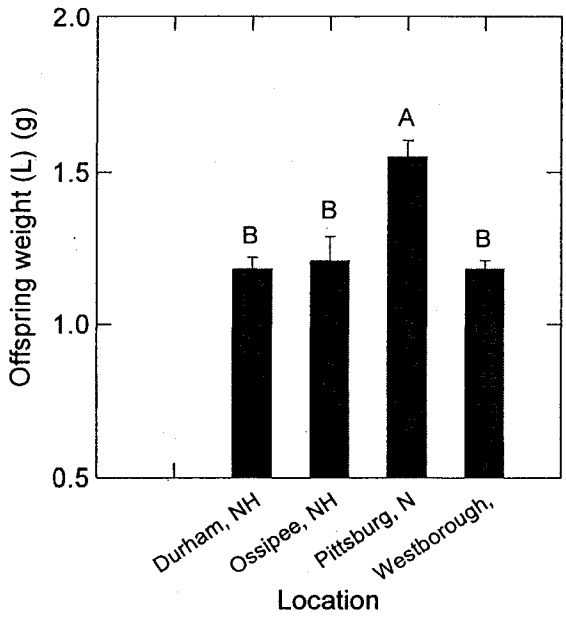


Figure 1.11. Mean offspring weight (g) versus location. This figure shows differences in mean weight of offspring *Thamnophis sirtalis* garter snakes from four populations in New England. Absolute values are represented, however tests for differences were completed using natural logarithm transformed values. The data set used to complete this analysis consisted of only live offspring counted.

Days in Captivity Effects

Table 1.9 shows a series of ANCOVA tests on effects of time a given pregnant female snake was held captive in the lab on postpartum morphometric measurements. The following six tests were the only parameters thought to be affected by days in captivity, as parameters such as clutch size, female snout-vent length, and female prepartum weight would have already been determined prior to capture and captivity.

Table 1.9 : ANCOVA results for laboratory effects

The following statistical tests were completed using a natural logarithm permutation for any variable which included weight or length.

Variable											
Relative clutch mass											
Source	DF	Sum of Squares	Mean Square	F Ratio	Source	DF	Sum of Squares	F Ratio	**Prob > F**	Figure	Pair-wise slope table
Model	7	0.05521	0.00789	0.3514	Days in captivity	1	0.00316	0.1407	**0.7085**	1.12	
Error	86	1.93023	0.02245	**Prob > F**	Location	3	0.02351	0.3491	**0.7899**		
C. Total	93	1.98544		**0.9275**	Days in captivity*Location	3	0.01859	0.2762	**0.8425**		
Female postpartum weight (g)											
Model	7	2.23028	0.31861	2.5295	Days in captivity	1	0.01191	0.0945	**0.7592**	1.13	
Error	87	10.9585	0.12596	**Prob > F**	Location	3	0.87907	2.3263	**0.0803**		
C. Total	94	13.1887		**0.0204**	Days in captivity*Location	3	0.32007	0.847	**0.4719**		
Mean Offspring weight (L+S) (g)											
Model	7	1.24712	0.17816	6.1628	Days in captivity	1	0.00285	0.0987	**0.7541**	1.14	
Error	87	2.51507	0.02891	**Prob > F**	Location	3	0.54775	6.3158	**0.0006**		
C. Total	94	3.76219		**<.0001**	Days in captivity*Location	3	0.10257	1.1827	**0.3211**		
Mean Offspring weight (L) (g)											
Model	7	1.32733	0.18962	7.9219	Days in captivity	1	0.00285	0.1191	**0.7309**	1.15	
Error	84	2.01063	0.02394	**Prob > F**	Location	3	0.60287	8.3956	**<.0001**		
C. Total	91	3.33796		**<.0001**	Days in captivity*Location	3	0.07439	1.0359	**0.381**		
Mean Offspring snout-vent length (L+S) (cm)											
Model	7	0.24489	0.03498	10.1123	Days in captivity	1	0.00036	0.1042	**0.7477**	1.16	1.8
Error	86	0.29752	0.00346	**Prob > F**	Location	3	0.08989	8.6613	**<.0001**		
C. Total	93	0.5424		**<.0001**	Days in captivity*Location	3	0.04316	4.1584	**0.0084**		
Mean Offspring snout-vent length (L) (cm)											
Model	7	0.24983	0.03569	10.6924	Days in captivity	1	0.00054	0.1614	**0.6889**	1.17	1.9
Error	84	0.28038	0.00334	**Prob > F**	Location	3	0.09724	9.711	**<.0001**		
C. Total	91	0.53021		**<.0001**	Days in captivity*Location	3	0.04453	4.4472	**0.006**		
Female postpartum weight (g)											
Model	7	2.23028	0.31861	2.5295	Days in captivity	1	0.01191	0.0945	**0.7592**	1.18	
Error	87	10.9585	0.12596	**Prob > F**	Location	3	0.87907	2.3263	**0.0803**		
C. Total	94	13.1887		**0.0204**	Days in captivity*Location	3	0.32007	0.847	**0.4719**		

No significant relationship was found between days in captivity and relative clutch mass (DF = 1, F= 0.1407, P = 0.7085) or location (DF = 3, F = 0.3491, P = 0.7899) (Table 1.9, Figure 1.12). Female postpartum weight showed no significant relationship with days in captivity (DF =1, F = 0.0945, P = 0.7592) or location (DF = 3, F = 2.3263, P = 0.0803) (Table 1.9, Figure 1.13).

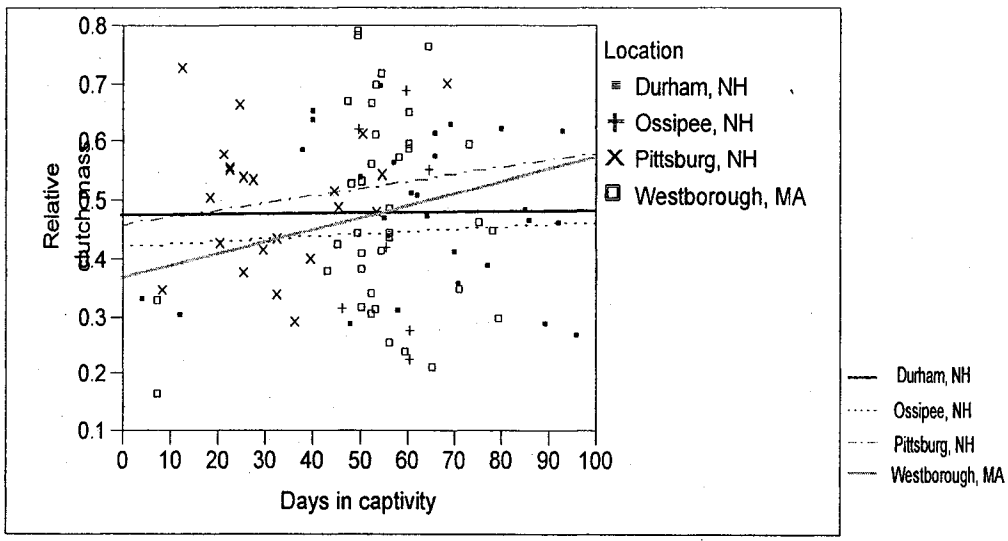


Figure 1.12. Relative clutch mass versus days in captivity. This regression plot was used to determine if days spent in captivity had any influence on the relative clutch masses.

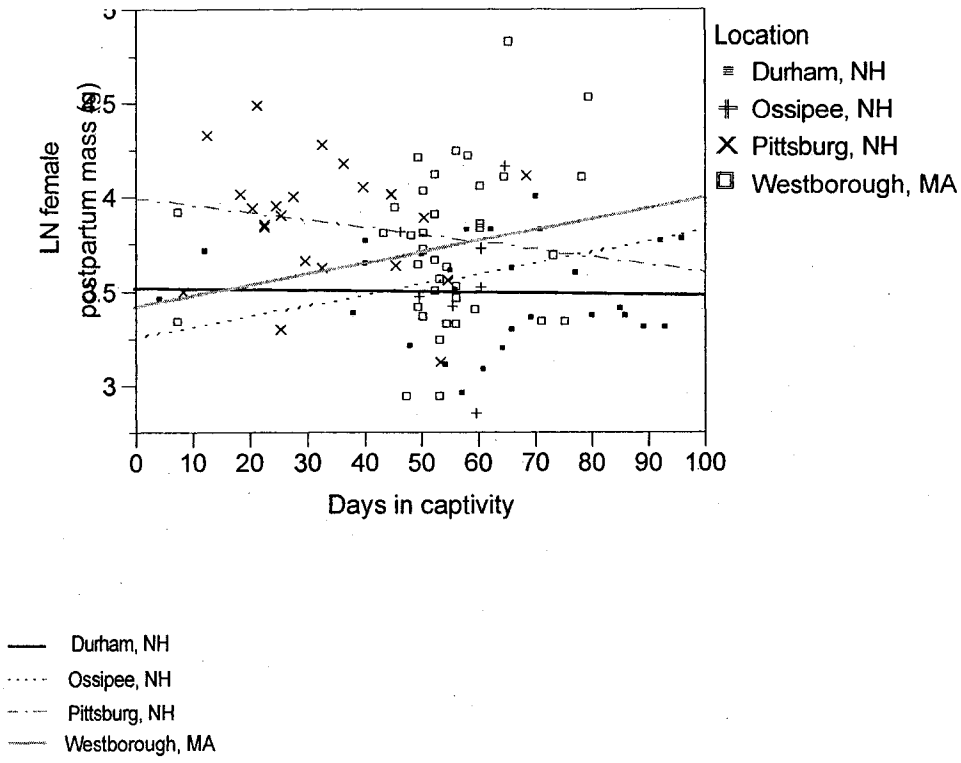


Figure 1.13. LN female postpartum weight (g) versus days in captivity. This regression was used to determine if days in captivity had any influence on the postpartum weight of female *Thamnophis sirtalis* individuals.

Offspring weight was not significantly affected by days in captivity, whether all offspring were used in the analysis (DF = 1, F = 0.0987, $P = 0.7541$) or only live-offspring were used in the analysis (DF = 1, F = 0.1191, $P = 0.7309$), but differences were noticed among locations (DF = 3, F= 6.3158, $P = 0.0006$; DF = 3, F = 8.3956, $P < 0.0001$) (Table 1.9, Figures 1.14, 1.15). These differences can be observed in Figures 1.10 and 1.11.

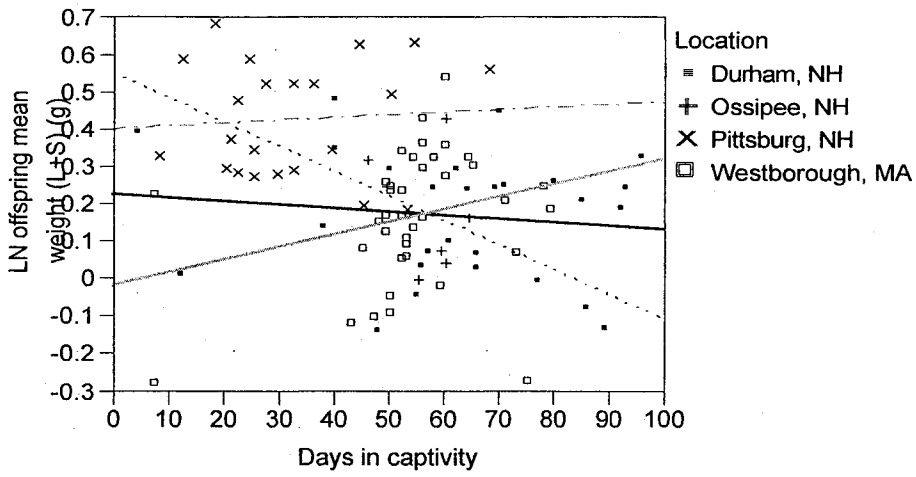


Figure 1.14. LN mean offspring weight (g) versus days in captivity. This regression was used to determine if days in captivity prior of female snakes to birth had any influence on the mean weight of the resulting offspring *Thamnophis sirtalis* snakes. The data set used for this analysis consisted of all offspring counted, live and still-born.

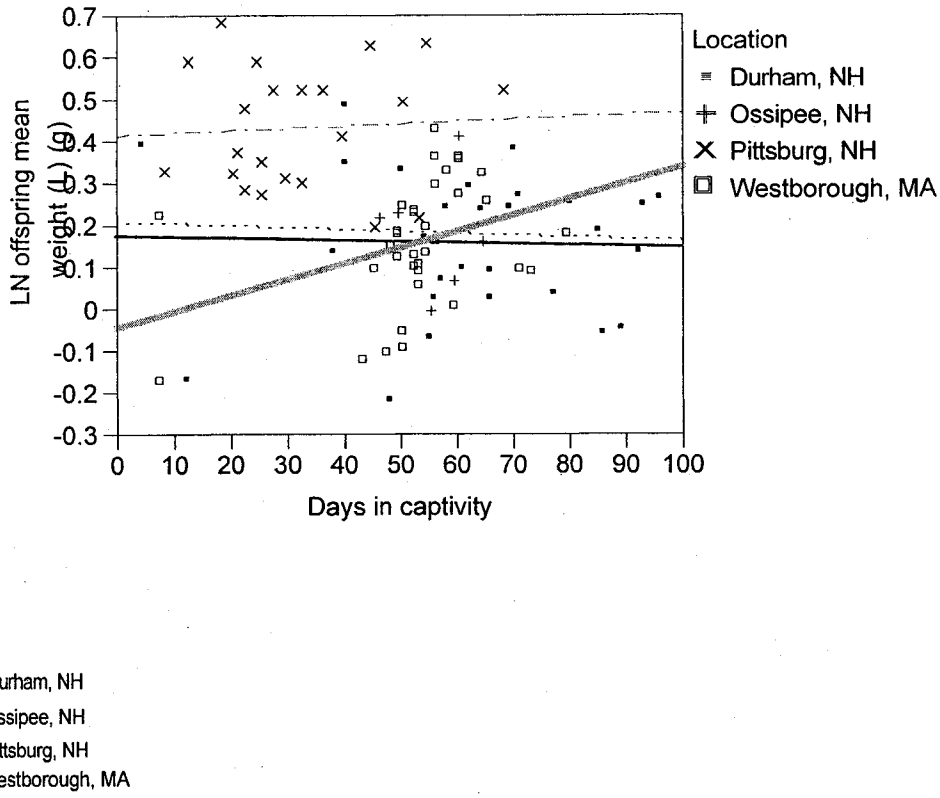


Figure 1.15. LN mean offspring weight (g) versus days in captivity. This regression was used to determine if days in captivity of the pregnant female garter snake had any influence on the mean weight of her offspring snakes. The data set used for this analysis consisted of only live offspring at time of birth.

A significant interaction was observed between the location and days in captivity regarding offspring snout-vent lengths (DF = 3, F = 4.1584, $P = 0.0084$; DF = 4.4472, F = 4.4472, $P = 0.006$) (Table 1.9, Figures 1.16, 1.17) signifying differences were observed among slopes in this relationship. Pair-wise tests for differences in slopes found a significant difference between the Durham and Westborough populations, and also Durham and Pittsburg (live-only data) (Tables 1.10, 1.11). All other comparisons were not significant.

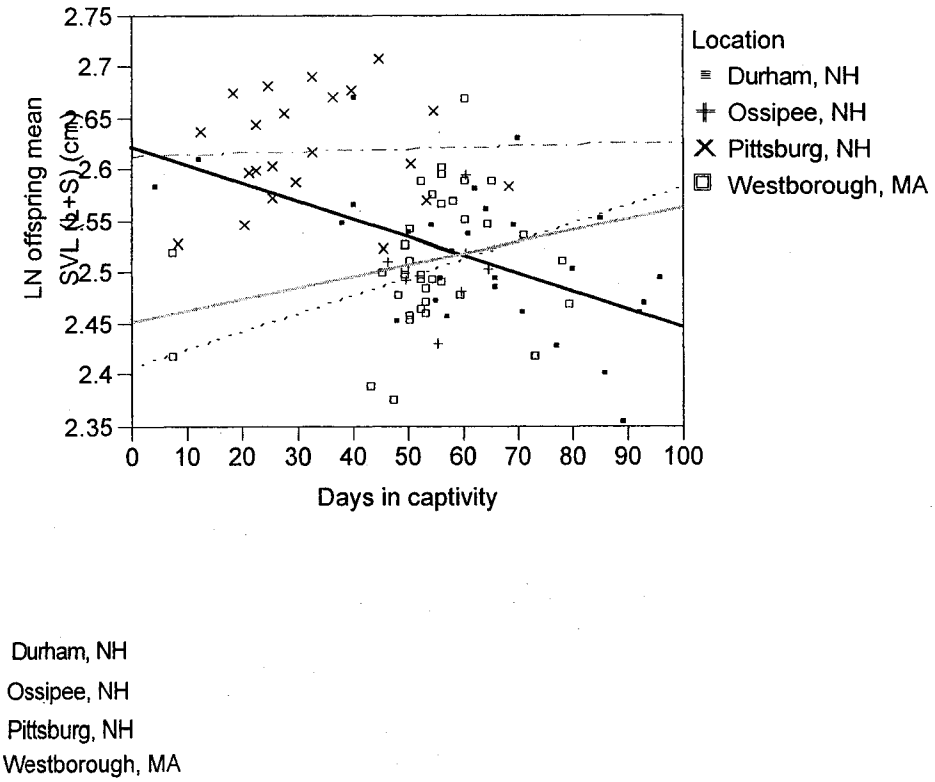


Figure 1.16. LN mean offspring snout-vent length (cm) (L+S) versus days in captivity. This regression plot shows the relationship between days spent in captivity of a pregnant female *Thamnophis sirtalis* garter snake, and the resulting mean snout-vent length of her clutch. The data-set used for this regression consisted of all offspring counted at time of birth, live and still-born.

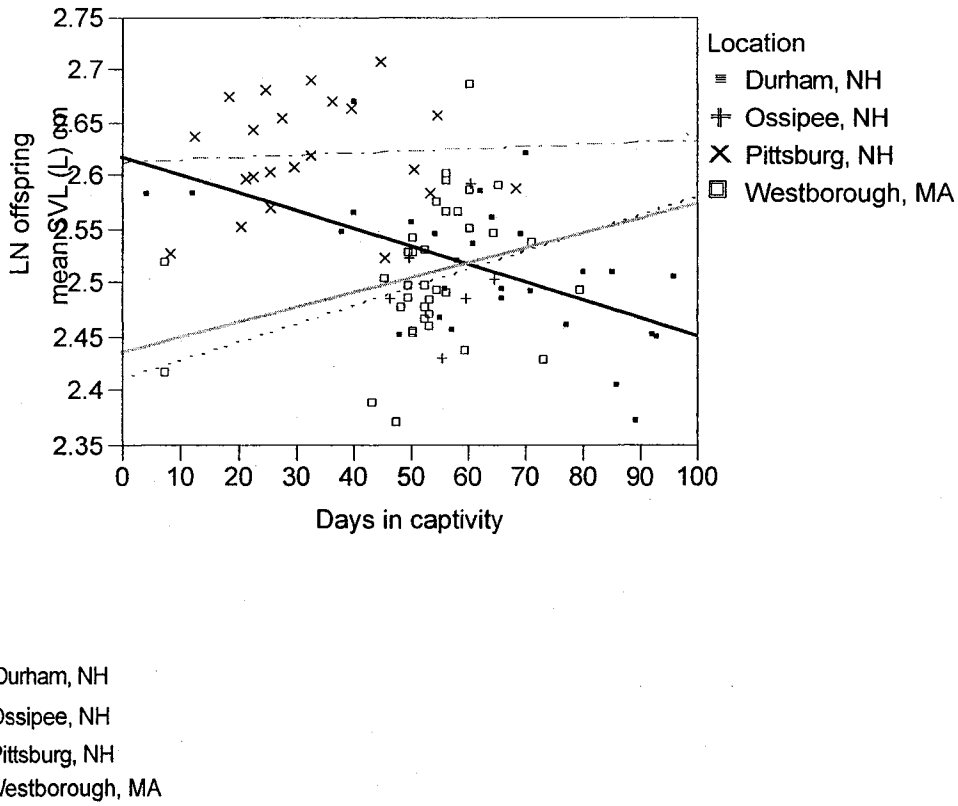


Figure 1.17. LN mean offspring snout-vent length (cm) versus days in captivity. This regression plot shows the relationship between days spent in captivity of a pregnant female *Thamnophis sirtalis* garter snake, and the resulting mean snout-vent length of her clutch. The data-set used for this regression consisted of only live offspring counted at time of birth.

Table 1.10 : Pairwise slope tests for LN mean offspring snout-vent length (cm) (L+S) vs. Days in captivity

Location	Slope (*Days in captivity)		Westborough, MA	Durham, NH	Ossipee, NH
Westborough, MA	0.0011	Westborough, MA	X	X	X
Durham, NH	-0.00176	Durham, NH	$P = 0.0019$*	X	X
Ossipee, NH	0.00175	Ossipee, NH	$P = 0.8729$	$P = 0.3448$	X
Pittsburg, NH	0.00014	Pittsburg, NH	$P = 0.3800$	$P = 0.0546$	$P = 0.6494$

* denotes significant differences

Table 1.11 : Pairwise slope tests for LN mean offspring snout-vent length (cm) (L) vs. Days in captivity

Location	Slope (*Days in captivity)		Westborough, MA	Durham, NH	Ossipee, NH
Westborough, MA	0.00137	Westborough, MA	X	X	X
Durham, NH	-0.00166	Durham, NH	$P = 0.0013$*	X	X
Ossipee, NH	0.00168	Ossipee, NH	$P = 0.9396$	$P = 0.3575$	X
Pittsburg, NH	0.00019	Pittsburg, NH	$P = 0.3016$	$P = 0.0449$*	$P = 0.6780$

* denotes significant differences

Body-Size Regressions

To test whether larger snakes tended to invest more of their body weight in offspring than smaller snakes, I regressed relative clutch mass versus (LN) female snout-vent length (cm). No significant interaction was observed for any of the populations (Table 1.12, Figure 1.18).

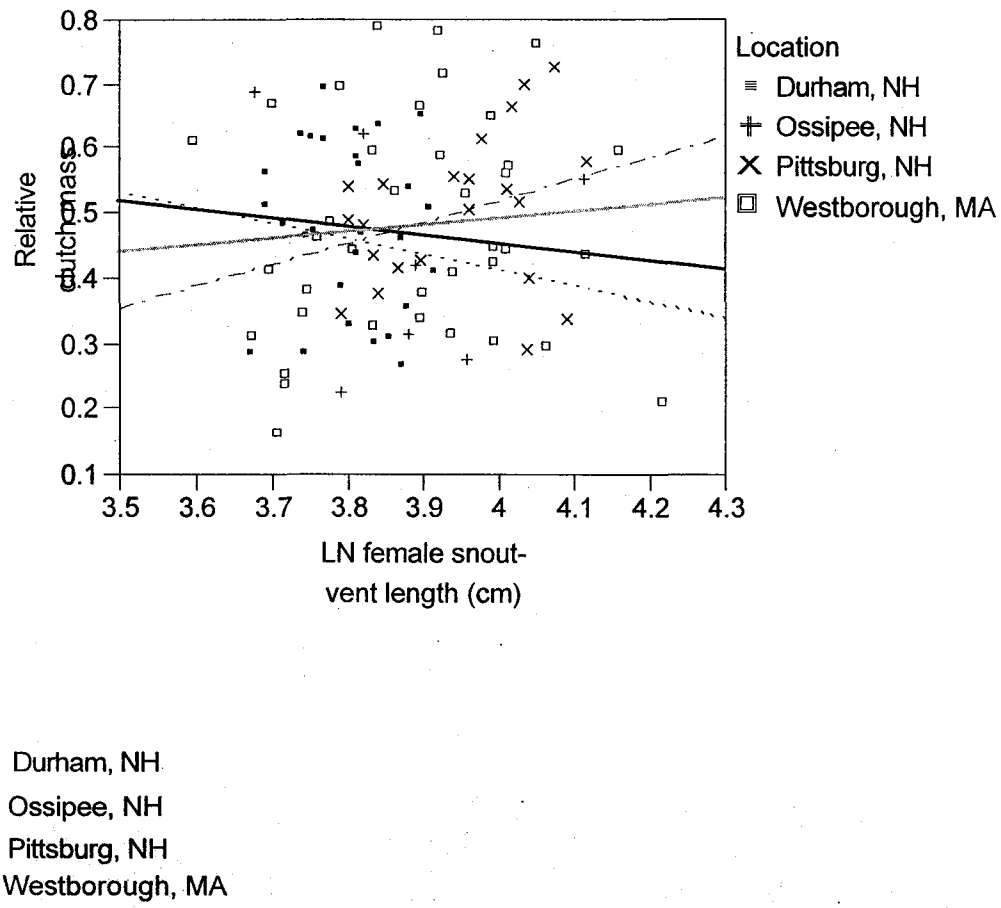


Figure 1.18. Relative clutch mass versus LN female snout-vent length (cm). This regression plot shows the relationship between length of a female snake, and a calculated relative clutch mass.

Table 1.12 : Regressions of body-size relationships

Regression

Relative clutch mass vs. LN Female snout-vent length (cm)

Analysis of Variance					**Effects**						
Source	DF	Sum of Squares	Mean Square	F Ratio	Source	DF	Sum of Squares	F Ratio	Prob > F	Adj. R Square	Figure
Model	7	0.0583	0.0083	0.3717	LN female snout-vent length (cm)	1	4.74E-05	0.002	**0.9634**	-0.04964	1.18
Error	86	1.92714	0.0224	**Prob > F**	Location	3	0.006044	0.09	**0.9654**		
C. Total	93	1.98544		**0.9165**	LN female snout-vent length (cm)*Location	3	0.032244	0.48	**0.6973**		

LN female postpartum weight (g) vs. LN female snout-vent length (cm)

Model	7	11.0234	1.5748	63.2716	LN female snout-vent length (cm)	1	5.929857	238.3	**<.0001**	0.822609	1.19
Error	87	2.16535	0.0249	**Prob > F**	Location	3	0.140628	1.883	**0.1383**		
C. Total	94	13.1887		**<.0001**	LN female snout-vent length (cm)*Location	3	0.104293	1.397	**0.2492**		

LN mean offspring snout-vent length (cm) (L+S) vs. LN female snout-vent length (cm)

Model	7	0.28017	0.04	13.1257	LN female snout-vent length (cm)	1	0.04275	14.02	**0.0003**	0.477175	1.20
Error	86	0.26224	0.003	**Prob > F**	Location	3	0.093484	10.22	**<.0001**		
C. Total	93	0.5424		**<.0001**	LN female snout-vent length (cm)*Location	3	0.016252	1.777	**0.1577**		

Table 1.12 (continued)

Analysis of Variance					**Effects**						
Source	DF	Sum of Squares	Mean Square	F Ratio	Source	DF	Sum of Squares	F Ratio	Prob > F	Adj. R Square	Figure
LN mean offspring weight (cm) vs. LN female postpartum weight (g)											
Model	7	1.72473	0.2464	10.5209	LN female postpartum weight (g)	1	0.329853	14.08	**0.0003**	0.414864	1.21
Error	87	2.03746	0.0234	**Prob > F**	Location	3	0.58815	8.371	**<.0001**		
C. Total	94	3.76219		**<.0001**	LN female postpartum weight (g)*Location	3	0.027057	0.385	**0.764**		
LN mean offspring weight (g) vs. LN female snout-vent length (cm)											
Model	7	1.94723	0.2782	13.3344	LN female snout-vent length (cm)	1	0.354596	17	**<.0001**	0.478764	1.22
Error	87	1.81496	0.0209	**Prob > F**	Location	3	0.520168	8.311	**<.0001**		
C. Total	94	3.76219		**<.0001**	LN female snout-vent length (cm)*Location	3	0.164338	2.626	**0.0554**		
LN mean offspring weight (g) vs. LN mean offspring snout-vent length (cm)											
Model	7	2.69261	0.3847	40.2215	Location	3	0.024658	0.859	**0.4654**	0.746973	1.23
Error	86	0.82246	0.0096	**Prob > F**	LN mean SVL (cm)	1	0.780264	81.59	**<.0001**		
C. Total	93	3.51508		**<.0001**	LN mean SVL (cm)*Location	3	0.034246	1.194	**0.3171**		

Figure 1.19 shows LN female postpartum weight (g) regressed against LN female snout-vent length (cm). A significant increasing trend is observed, suggesting longer females are heavier (DF= 1, F = 232.252, $P < 0.0001$). No differences were calculated among populations (DF = 3, F = 1.8834, $P = 0.1383$) (Table 1.12, Figure 1.19).

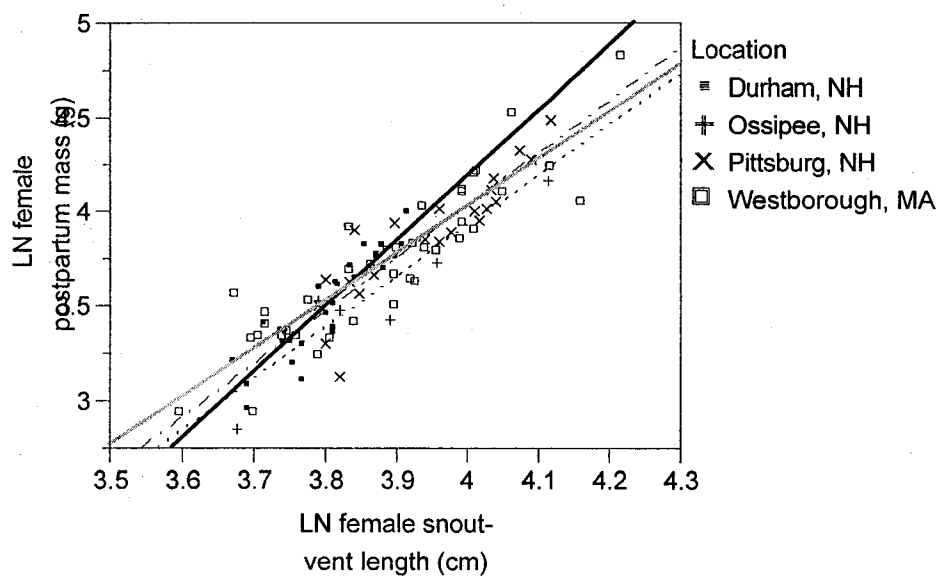
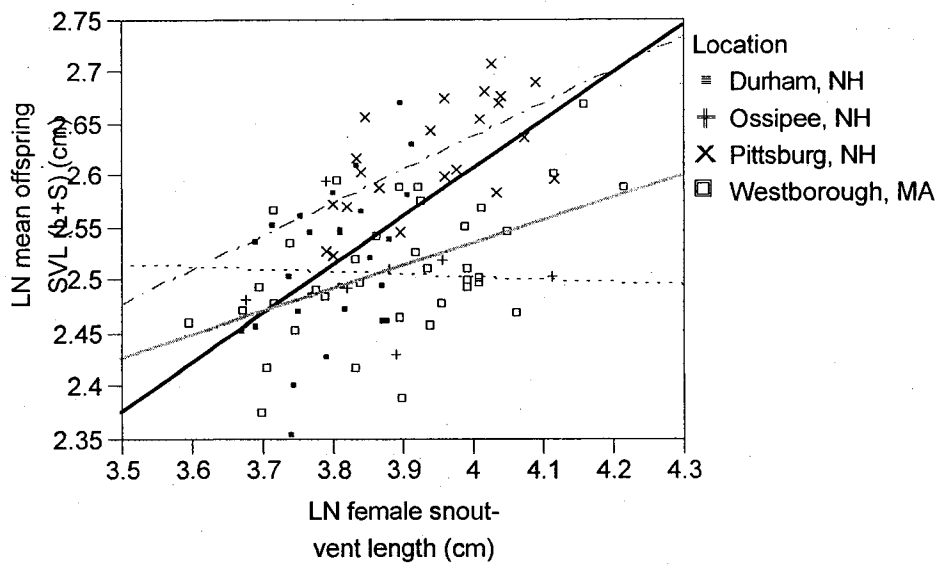


Figure 1.19. LN female postpartum weight (g) versus LN female snout-vent length (cm). This regression shows the relationship between length of a female *Thamnophis sirtalis* garter snake, and her weight after birth.

Regressing LN mean offspring snout-vent length (cm) versus LN female snout-vent length (cm), showed a significant positive relationship (DF = 1, F = 14.0196, $P = 0.0003$), showing that larger females have longer offspring (Table 1.12, Figure 1.20).

Differences were also observed among populations (DF = 3, F = 10.2193, $P < 0.0001$) (Table 1.12, Figure 1.20). Pittsburg offspring were significantly longer than the other three populations (Figure 1.8).



Durham, NH
Ossipee, NH
Pittsburg, NH
Westborough, MA

Figure 1.20. LN mean offspring snout-vent length (cm) versus LN female snout-vent length (cm).

Regressing LN mean weight (g) of offspring versus LN postpartum weight (g) of females showed a significant positive relationship, suggesting heavier females give birth to heavierr offspring (DF = 1, F = 14.0848, $P = 0.0003$) (Table 1.12, Figure 1.21). In addition, differences in this relationship were also observed among populations (DF = 3, F = 8.3714, $P < 0.0001$) (Table 1.12, Figure 1.21). Figure 1.10 shows the Pittsburg, NH offspring were significantly heavier than the other three populations.

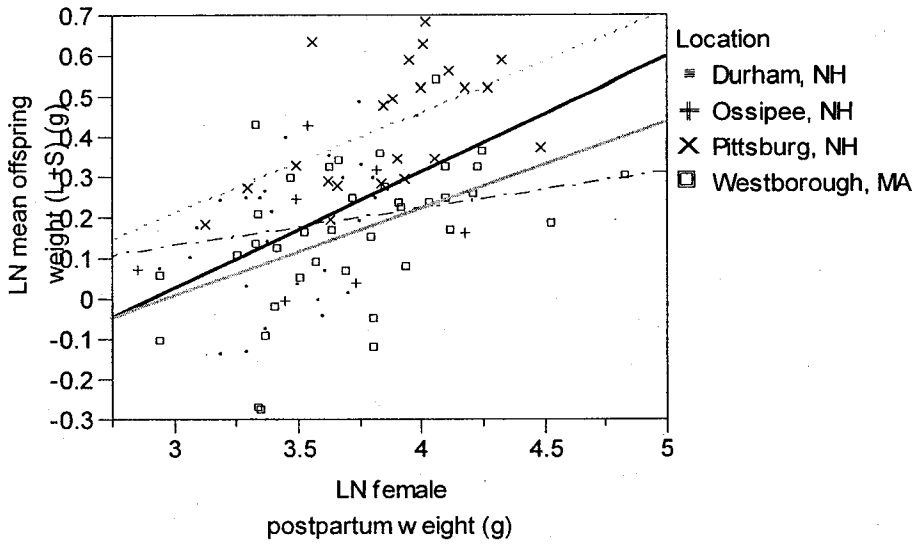


Figure 1.21. LN mean offspring weight (g) versus LN female postpartum weight (g).

Figure 1.22 shows the results of regressing LN mean offspring weight (g) versus LN snout-vent length (cm) of female. The trends in this chart suggest a general increasing in the weight of offspring as length of female increases (Table 1.12, Figure 1.22). This relationship was also significant (DF = 1, F = 16.9975, $P < 0.0001$) and different among populations (DF = 3, F = 8.3114, $P < 0.0001$). Again Pittsburg, NH offspring were significantly heavier than the other three populations (Figure 1.10).

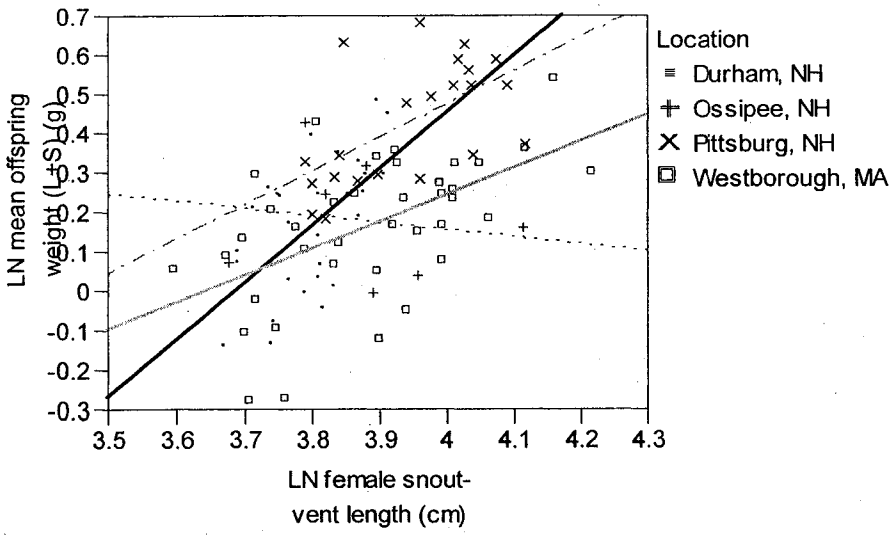


Figure 1.22. LN mean offspring weight (g) versus LN female snout-vent length (cm).

Mean weight (g) of offspring versus mean snout-vent length (cm) of offspring, showed a significant increasing relationship suggesting longer offspring are heavier than shorter offspring (DF = 1, F = 81.5876, $P < 0.0001$). No significant differences were observed among locations in this relationship (DF = 3, F = 0.8594, $P = 0.4654$) (Table 1.12, Figure 1.23).

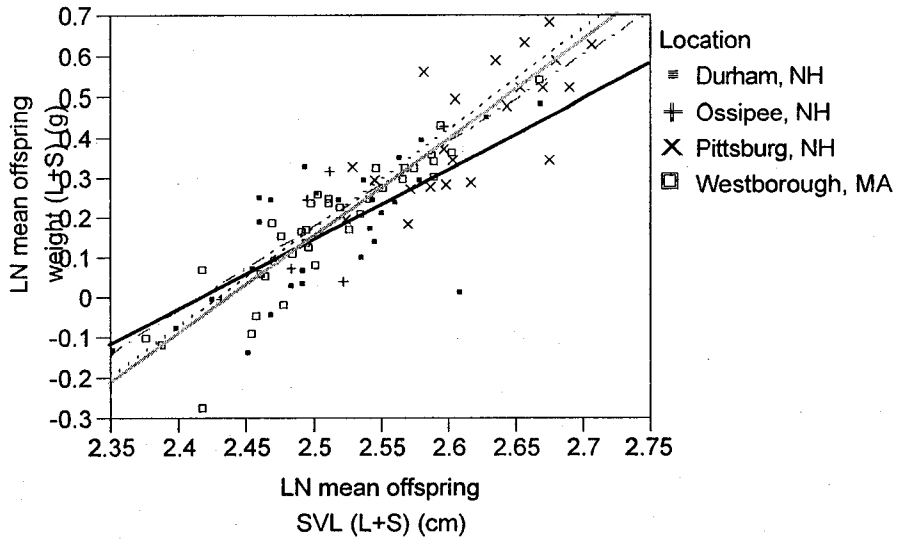


Figure 1.23 LN mean offspring weight (g) versus LN mean offspring snout-vent length (cm).

Regressions to Test for Reproductive Trade-Offs

Figure 1.24 shows LN mean offspring weight (g) versus clutch size. Despite significant differences among locations (DF = 3, F = 13.1502, $P < 0.0001$), no significant relationship was observed between mean weights of offspring and clutch size (DF = 1, F = 1.012, $P = 0.3172$) (Table 7). Differences in mean weights of offspring are shown in Figure 1.10 as Pittsburg, NH snakes were heavier than the other three populations.

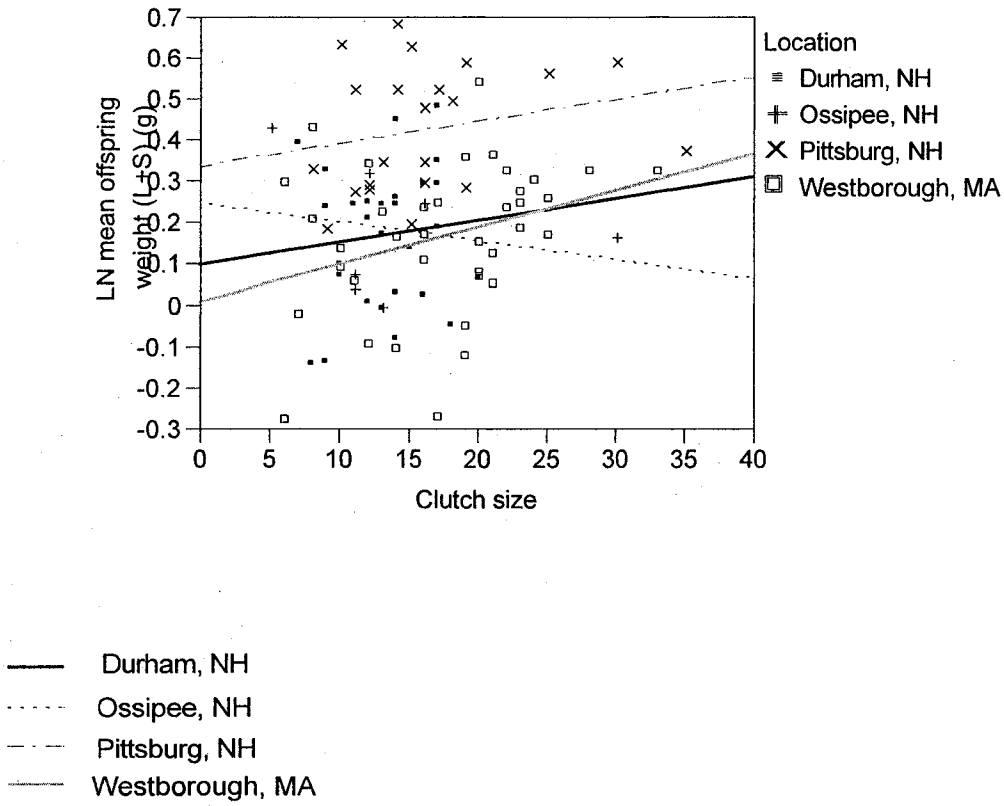


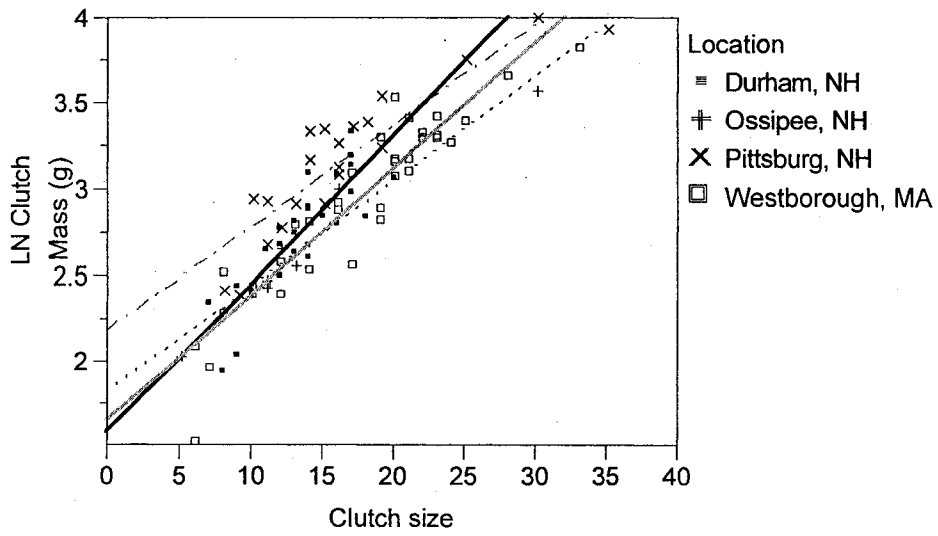
Figure 1.24. LN mean offspring weight (g) versus clutch size.

Table 1.13. Tests for trade-offs in reproductive output.

LN mean offspring weight (g) (L+S) vs Clutch size

Analysis of Variance					**Effect Tests**							Pairwise
Source	DF	Sum of Squares	Mean Square	F Ratio	Source	DF	Sum of Squares	F Ratio	Prob > F	Adj. R-square	Figure	Slope Table
Model	7	1.298996	0.185571	6.5544	Location	3	1.1169454	13.1502	**<.0001**	0.293	1.24	
Error	87	2.4631958	0.028313	**Prob > F**	Clutch size	1	0.028653	1.012	**0.3172**			
C. Total	94	3.7621918		**<.0001**	Clutch size*Location	3	0.0530252	0.6243	**0.6012**			
LN Clutch mass vs. Clutch size												
Model	7	17.451949	2.49314	78.9746	Location	3	1.500156	15.8401	**<.0001**	0.853	1.25	
Error	87	2.746489	0.03157	**Prob > F**	Clutch size	1	9.8997221	313.592	**<.0001**			
C. Total	94	20.198437		**<.0001**	Clutch size*Location	3	0.2314641	2.444	**0.0694**			
Relative clutch mass vs. Clutch size											1.26	
Model	7	0.6434431	0.09192	5.9217	Location	3	0.08213634	1.7638	**0.16**	0.323		
Error	87	1.3504735	0.015523	**Prob > F**	Clutch size	1	0.4512039	29.0674	**<.0001**			
C. Total	94	1.9939167		**<.0001**	Location*Clutch size	3	0.02884801	0.6195	**0.6043**			
LN Clutch mass vs. Residuals of LN female snout-vent length and LN female prepartum weight											1.27	1.14
Model	7	4.582093	0.654585	3.6467	Residual LN female snout-vent length (cm)	1	0.7481773	4.1682	**0.0442**	0.164		
Error	87	15.616344	0.179498	**Prob > F**	Location	3	3.4540204	6.4142	**0.0006**			
C. Total	94	20.198437		**0.0017**	Residual LN female snout-vent length (cm)*Location	3	1.7053741	3.1669	**0.0284**			

Regressing LN clutch mass versus clutch size (Figure 1.25), showed a significant positive relationship suggesting higher clutch sizes had higher clutch masses (DF = 1, F = 313.5916, $P < 0.0001$, Adjusted R-square = 0.853). Significant differences were observed among locations (DF = 3, F = 15.8401, $P < 0.0001$) (Table 1.13). Figure 1.5 shows that clutch masses were highest in Pittsburg, NH, and lowest in Durham, NH.



Durham, NH
Ossipee, NH
Pittsburg, NH
Westborough, MA

Figure 1.25. LN clutch mass (g) versus clutch size.

Regressions of relative clutch mass against clutch size are shown in Figure 1.26. No significant differences were observed among populations (DF = 3, F = 1.7638, $P = 0.16$), however a significant positive relationship was observed between relative clutch

mass and clutch size suggesting larger clutch masses were correlated with higher RCM (DF = 1, F = 29.0674, $P < 0.0001$, Adjusted R-Square = 0.328) (Table 1.13).

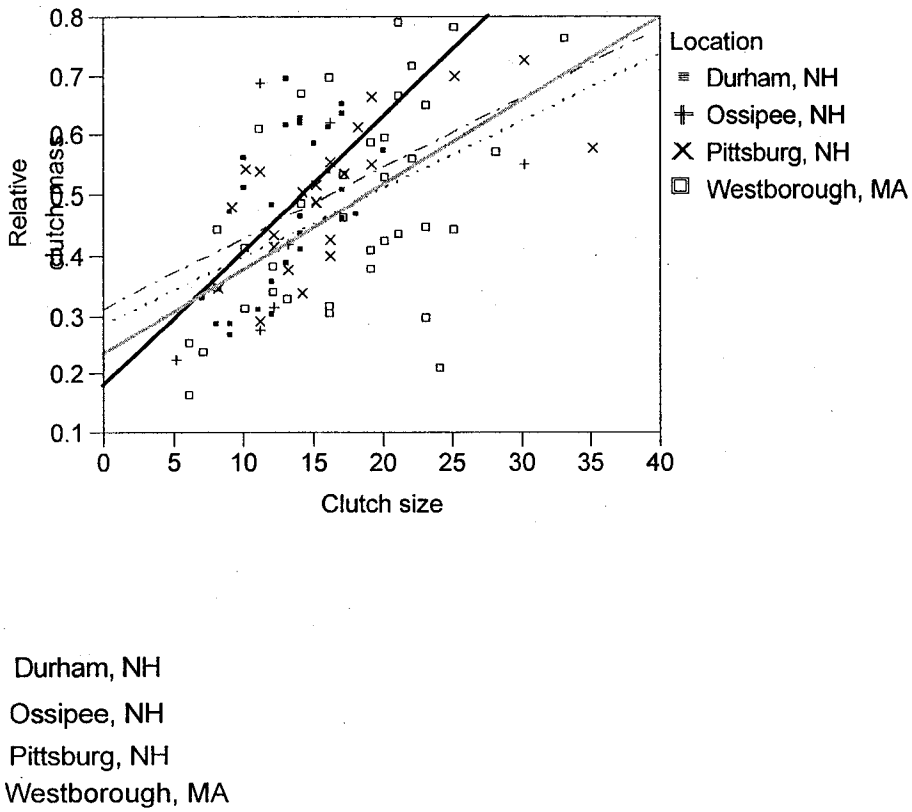


Figure 1.26. Relative clutch mass versus clutch size. This set of regressions shows the relationship between relative clutch mass and clutch size.

Figure 1.27 shows LN clutch mass versus residuals of LN female snout-vent length and LN female prepartum mass. A significant interaction was observed between these variables and the location covariate. Pair-wise tests for differences in slopes found significant differences between Pittsburg, NH and Westborough, MA, and also Durham, NH and Westborough, MA (Table 1.14). The negative slopes in Durham and Pittsburg populations would suggest better conditioned females have smaller clutch masses than poorer-conditioned females. However, the positive slope of Westborough would suggest the opposite.

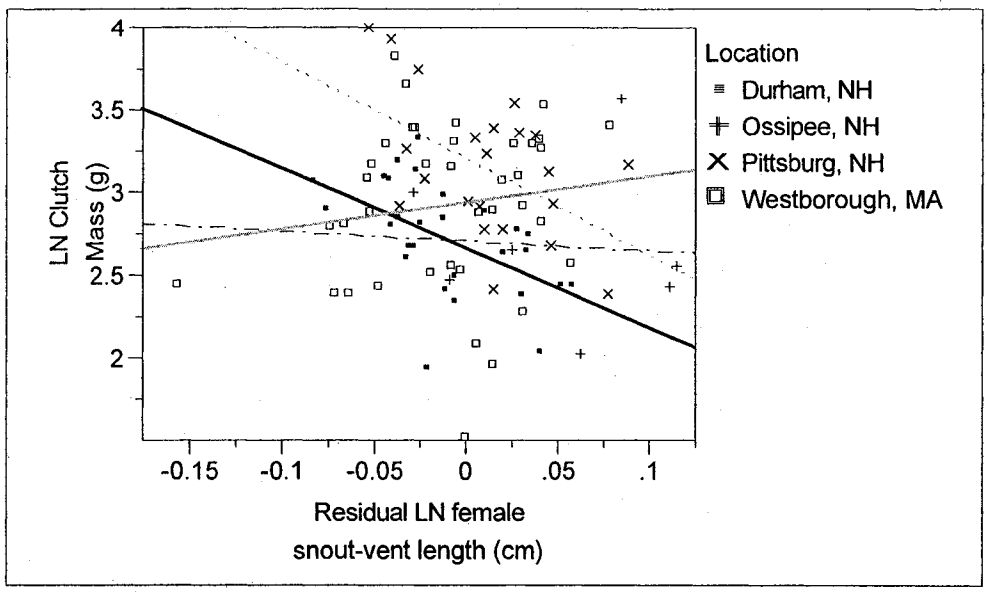


Figure 1.27. LN Clutch mass versus residuals of LN Female snout-vent length (cm) and LN Female prepartum weight (g).

Table 1.14 : Pairwise slope tests for LN Clutch Mass vs. Residuals of LN Female snout-vent length (cm) and LN Female prepartum weight (g)

Location	Slope (*residuals)		Westborough, MA	Durham, NH	Ossipee, NH
Westborough, MA	1.55	Westborough, MA	X	X	X
Durham, NH	-4.81	Durham, NH	$P = 0.0227$*	X	X
Ossipee, NH	-0.56	Ossipee, NH	$P = 0.6047$	$P = 0.1675$	X
Pittsburg, NH	-5.85	Pittsburg, NH	$P = 0.0230$*	$P = 0.6906$	$P = 0.1772$

* denotes significant differences

Discussion

Clutch Summaries and Reproductive Sizes for Female *Thamnophis sirtalis*

One goal of this study was to describe clutch characteristics and body sizes for four populations of *Thamnophis sirtalis* snakes within New England. The overall patterns show that snakes from New England grow and reproduce at similar sizes with similar clutch sizes as reported for other *T. sirtalis* populations from across the continent (Figures 1.2-1.6). Across all four populations, mean clutch sizes ranged from 13 to 17, which are within the range reported by other studies (Burt, 1928; Zehr, 1962; Cover and Boyer, 1988; Gregory and Larsen, 1993). One previous population studied in western New Hampshire yielded mean a mean clutch size of 12.9 (Zehr, 1962). Lengths of reproductive females captured during this study ranged from 39 to 67 cm (Appendix B). Most females mature at SVL's above 42 cm (Rossman et al., 1996), so this study documents smaller reproducing snakes in New England than elsewhere. Female *T. sirtalis* snakes captured on Georges Island, Nova Scotia showed reproductive activity at SVL's of 35cm (Barnes et al., 2006). These two recent reports (this study and Barnes et al. (2006)) add support to Gregory and Larsen's claim, that an east-west trend exists across North America in *T. sirtalis* reproductive output, as eastern snakes are smaller, and show larger clutch sizes for smaller snakes (Gregory and Larsen, 1993). They suggested genetic reasons for such a cline, but moisture availability and resulting availability of amphibians and other prey in Northern New England might allow for quicker reproductive development through smaller size classes.

Comparisons of Relative Clutch Mass

Relative clutch mass (RCM) is a useful measure of reproductive output, as it can be compared among taxa on many levels (i.e. order to species) and reproductive life histories, (viviparous versus oviparous) (Seigel and Fitch, 1984; Seigel et al., 1986). In previous work, it was shown that multiple species of small snakes yielded higher RCM, when compared to larger snakes (Seigel et al., 1986). This study with *Thamnophis sirtalis* showed insignificant trends in RCM when comparing populations (Table 1.5, Figure 1.7) or differences in body size (Figure 1.18). In general however, this study showed high RCM (40% to >50% (Table 1.5, 1.12, Figures 1.7, 1.18)) for snakes across all populations, as most documentation of RCM show *T. sirtalis* with RCM's below 30% (Seigel and Fitch, 1984; Seigel and Fitch, 1985). This could show further evidence that northeastern snakes are able to reproductively invest more in offspring than snakes of similar size elsewhere.

RCM is calculated by dividing the total mass of all offspring live and dead (clutch mass) by prepartum female mass. Conditions affecting either of these variables (total offspring mass or postpartum female mass) would influence RCM calculations. In previous studies of other northern viviparous snakes, populations with higher daily temperatures had lower postpartum masses (Lourdais et al., 2002b). Higher metabolic rates were hypothesized to result in lower masses. Higher metabolic rates could also alter prepartum weights, although higher temperatures also result in shorter gestation periods (Lourdais et al., 2002b). In this study, since no substantial trends are seen when RCM is plotted against days in captivity (Table 1.9, Figure 1.12), metabolic rates of pregnant female snakes should have been approximately constant throughout their captivity. I

attempted to control for differences in environmentally-induced metabolic changes by maintaining stable temperature-ranges, and feeding snakes constant amounts of food throughout captivity.

Geographic Trends in Female Sizes

This study yielded significant but inconclusive results about pregnant female size and location. Mean prepartum weight, postpartum weight, and snout-vent lengths were all highest or longest in Pittsburg females (Table 1.5, Figures 1.2-1.4). Based on Tukey-Kramer comparisons tests, these values were not significantly higher than populations found further south. Clutch sizes were shown to be highest in Westborough, MA populations, but not statistically higher than other populations (Figure 1.6). Females from the Durham, NH population were consistently the smallest of all the populations and also had the smallest and lightest clutches. This might indicate a length or size constraint within this localized population or a type I error resulting from incomplete sampling.

Geographic Trends in Offspring Size

A second goal of this study was to describe characteristics of offspring at time of birth from the four populations of snakes in New England. In all tests (Table 1.6, Figures 1.8-1.11) the Pittsburg population produced the largest and longest offspring. These results match those found by Larsen et al. (1993) that populations found near the northern limit of the species' range show larger offspring characters at time of birth.

No consistent geographic trend was observed between the three populations of snakes found further south. Small sample sizes, particularly in Ossipee, NH (N=8), may have limited statistical power. However, the lack of a consistent trend could point to

phenotypic plasticity among populations, and unless populations are under greater stress (i.e. as in near the limit of range) no trend may be observed in sizes.

I found no substantial differences between offspring attributes calculated with only live offspring data, or both live and dead offspring combined in the data set (Table 1.6, Figures 1.8-1.11). There was one pair-wise slope test which plotted offspring snout-vent lengths against days in captivity (Tables 1.10, 1.11), where there was a significant difference between the Durham population and Pittsburg population with only live offspring used ($P = 0.0449$) but only a nearly significant difference with all offspring counted ($P = 0.0546$). These results contradict the findings of Gregory and Farr (1991). Gregory and Farr saw different findings in offspring trends when they used only live offspring, versus all live and dead offspring in their analyses (Farr and Gregory, 1991). Their study involved a different, but related species *Thamnophis elegans*. In addition, they palped females post-parturition to release any undeveloped eggs, which may have added an extra number to clutch size (Farr and Gregory, 1991). Females in my study yielded still-born offspring, but no undeveloped eggs after natural parturition, (W. Kean personal observation). Some offspring may have remained uncounted, as I did not induce undeveloped eggs to emerge.

Effects of Laboratory

Previous researchers have warned not to maintain pregnant female snakes in laboratory for long periods of time, as the effects (stress, feeding regime, laboratory temperatures etc.) might affect results in female and offspring size and condition (King, 1993b). This study did not find any significant evidence that time spent in captivity prepartum, influenced the weight of the offspring, postpartum weight of female, or

relative clutch mass (Table 1.9, Figures 1.12-1.15). Only SVL of offspring from Durham, NH showed a significant decreasing trend with increased time spent in lab (Tables 1.10- 1.11, Figure 1.17). Evidence of laboratory effects had come from other species, which may have been reasoning for a difference in results. I think also this study with *T. sirtalis* had been controlled amply with influencing variables such as laboratory temperatures, feeding regime, and water availabity.

Reasons for Geographic Trends

Although his study has documented the presence of some geographic clines in reproductive attributes of *Thamnophis sirtalis* garter snakes, the underlying reasons for these patterns remain more difficult to describe. Developmental temperature from time of capture through parturition, and nutritional inputs from time of capture through parturition were constant throughout this study. These factors are key components to offspring development of many ectothermic viviparous animals, especially reptiles. Because *T. sirtalis* is thought to be primarily a capital-breeding species (energy for development comes from energy reserves), nutritional inputs during gestation, particularly later in development should have been inconsequential for attributes we were monitored (Ford and Seigel, 1989; Gregory and Skebo, 1998). Nonetheless, we attempted to keep temperature and nutrition variables constant.

One possible reason for larger female snakes and resulting offspring being found further north, was a difference in capture techniques: snakes captured in the Pittsburg, NH site were captured exclusively by visual spotting and hand-capture, and in other locations, cover objects were employed to passively attract snakes. Because larger snakes are easier to spot from a distance, and heavier snakes are less likely to escape

capture (Seigel et al., 1987; Bonnet and Naulleau, 1996) it is possible that the Pittsburg, NH sample may have been biased towards larger, heavier snakes. Moreover, in other locations where cover objects were used to attract snakes, sampling may have been less biased towards large snakes, as all pregnant snakes search out warmer spots during pregnancy. However, I believe this difference with collection methods should not have skewed results, as approximately 30-40 smaller juvenile and male snakes were also captured and released at the Pittsburg, NH site during field collection (W. Kean personal observation).

Another hypothesized reason for larger snakes being found further north, particularly offspring, is that snakes are under higher survival pressures further north due to fewer frost-free days, and lower daily summer temperatures, and earlier frosts in first months post-partum. Associated with this is the fact that larger snakes might be less likely to freeze to death due to higher volume than smaller snakes. Therefore, only larger snakes can survive to sexual maturity and reproduce. Because larger snakes yield larger offspring (Table 1.12, Figures 1.20-1.22), this survival "wall" could yield larger snakes further north. If only larger snakes are surviving to reproduction, there could be a selective-genetic component to this geographic trend as well. It is possible that larger offspring are born, and develop faster and larger than other offspring populations, because generations of only larger adults had survived. In locations further south, this selective-genetic pressure may not be present.

As stated previously (see geographic trends), adult snakes from Durham, NH were consistently shown to have smaller body sizes than the other three populations. The study site in Durham, NH was primarily a wet meadow square in shape, about 150 meters

per side and surrounded by dense-canopy forest. This habitat might be ideal for bird predators to attack snakes as there are ample "perch and search" locations surrounding the sampling location. Since large snakes have more difficulty escaping capture, and should be easier to spot from above, this might also be reason for why no larger size-class snakes were captured in Durham.

Differential predatory rates on *Thamnophis* snakes could be calculated in a few ways in future studies of these snake populations. Placyk and Burghardt (2005) counted scarring and tail "stubs" in *T. sirtalis* populations in mainland and island populations in the Northern Great Lakes to test for differential predatory rates. A similar study could be conducted here in the Northeast. Furthermore, as clay model snakes have been used to judge many aspects of crypsis and predatory rates on snakes, one could conduct a similar study in the Northeast with model *T. sirtalis* snakes of various sizes, and calculate differential rates of claw and beak marks in the clay models.

Reproductive Trade-Offs

This study also attempted to investigate if trade-offs existed within reproductive characteristics. There has to be some limit to how much energy each female can place into a single reproductive event. Evidence for some of these potentials trade-offs would be observed in negative slopes, or a plateau in the relationship when two variables (i.e. clutch mass versus mean offspring weight) were plotted, showing limits in reproductive output. All such statistical tests yielded no evidence of trade-offs (Figures 1.24-1.27). The residual plot (Figure 1.27) had some evidence that better-conditioned females had lower clutch masses, which might suggest they could better invest in future offspring. Likewise, it has been suggested that trade-offs might be better judged in these viviparous

snakes over longer time-scales, as survivorship of adult females should also be considered (Lourdais et al., 2002). In this study, all females which were released after birth in 2005 were PIT-tagged. In 2006, of the 23 females PIT-tagged in 2005, only four were recaptured, and none of these recaptured individuals yielded offspring in the second consecutive year (Table 1.4). Due to small sample size (N=4) it would be inappropriate to suggest that there is a temporal reproductive trade-off in New England *T. sirtalis* populations. However, these few examples do agree with other published studies, suggesting that *T. sirtalis* in northern populations does not reproduce in consecutive years (Ernst and Ernst, 2003).

Other Implications

Another pattern observed in these results, is that there are some significant differences in reproductive characters for the two proposed subspecies of *Thamnophis sirtalis* studied in this survey. The Eastern garter snake, *T. s. sirtalis*, is typically described as existing slightly north of the Ossipee, NH region, and all regions further South and West in New England (DeGraaf and Rudis, 1983; Tennant, 2003). The Maritime garter snake, *T. s. pallidulus*, is found north and east of the previously described range of *T. s. sirtalis*. This study only showed differences between offspring sizes across the studied populations. Taxonomic descriptions between the subspecies are almost exclusively based on morphological characters. Based on field observations, I would place all pregnant females from Pittsburg, NH as *T. s. pallidulus*, and all snakes found further south as *T. s. sirtalis* except for one snake in Ossipee. The second half of this thesis describes the mitochondrial DNA differences between these two proposed subspecies. However, implications that perhaps these two subspecies should be

described differently based on differences in reproductive measures are not supported strongly.

CHAPTER II

MOLECULAR PHYLOGEOGRAPHY OF *THAMNOPHIS SIRTALIS* IN NEW ENGLAND

Introduction

Geologic History of Region

The northeastern United States has experienced a series of glaciated time periods over the last two million years (Flint, 1957; Andrews, 1997). The most recent glaciation event happened at the end of the Pleistocene Era between 20,000-10,000 years BP, when the Laurentide Ice sheet covered all of the northeastern United States and the Great Lakes Region, as far south as central Indiana and Illinois (Flint, 1957). Radiocarbon dating suggests that parts of southern New England were ice-free 18,000 years BP, and almost the entirety of New England was ice free 12,000- 14,000 years BP (Andrews and Dyke, 2007). The melting glacial retreat left areas a few hundred miles north of the current Canadian- United States border, including all of the St. Lawrence Seaway ice-free 10,000 years BP(Andrews and Dyke, 2007). It is suggested that many species which may have existed in these regions before glaciation may have moved further south, or found refuges in small localized pockets of ice-free regions such as western Wisconsin during these glaciated time-periods (Holman, 1995).

Fossil evidence shows that a number of extant species of reptiles and amphibians existed in refuges during this glaciated time in regions of the midwestern and southern United States. These fossils suggest the starting locations of recolonizing founding

populations (Holman, 1995). *Thamnophis* species (including definitive evidence of *T. sirtalis)* have been found in at least three locations in the Midwest dating to times between 17,500 to 11,700 years BP (Hansen, 1992; Holman, 1994). These areas include: Sheriden Pit in northwestern Ohio (Holman, 1994); Prairie Creek in southwestern Indiana (Holman and Richards, 1993), and Moscow Fissure in southwestern Wisconsin (Foley, 1984). There are also fossil records of *Thamnophis* individuals from the Appalachian region as far north as Pennsylvania (Fay, 1984; 1988), and further south in southeastern United States from similar time periods (Meylan, 1984).

Post-glacier reinvasion by amphibian and reptile species likely happened as soon as habitats became tolerable for existence. Once glaciers receeded, permafrost would have also had to thaw before these ectothermic species could resettle. Only the most cold-adapted species, and those which could exist in coniferous forests, would have been among the first species to invade these glaciated regions (Holman, 1995). Pollen records show that coniferous forests containing pines (*Pinus*), spruce (*Picea*) and fir (*Abies*) constituted most of the plant communities immediately following retreat of glaciers (Godbout et al., 2005). *Thamnophis sirtalis* was probably one of the first snake species to invade newly-opened regions post-glaciation in the Northeast and elsewhere, due to its high cold-tolerance, and its resulting current wide-distribution in northern habitats (Larsen and Gregory, 1989).

This chapter focuses on phylogenetic reconstruction and the recolonization history of *Thamnophis sirtalis* snakes currently found in five locations in northern New England. The following introduction to phylogenetics, subspecies classifications, and

New England garter snakes will lead into a more specific description of research goals and hypotheses.

Mitochondrial Genome and its use in Molecular Phylogenetics

Animal mitochondrial DNA (mtDNA) has been used in a variety of population genetic- and evolutionary studies. MtDNA is thought to be relatively regular in mutational rate (at least within a given taxon), and almost all base-pairs within the circular mitochondrial genome code for a functional protein within the mitochondrion or whole cell (Avise, 2004). Most vertebrate mitochondrial genomes are between 15,000-17,000 base-pairs in length and code for 13 protein-coding genes, 22 tRNA genes, and two ribosomal subunits (Graur and Li, 2000; 1982). Regions used for sequencing and subsequent phylogenetic reconstruction often include the seven subunits of the NADH-upiquinone oxireductase pathway, or cytochrome *b* regions (Graur and Li, 2000). Cytochrome *b* is included in the mitochondrial oxidative phosphorylation system, and is the only one of the associated proteins which actually is coded within the mitochondrial genome (Hatefi, 1985; Irwin et al., 1991). The animal mitochondrial genome is known to have a relatively high mutational rate for three reasons: low fidelity of DNA replication mechanisms, inefficient repair mechanisms, and high concentrations of mutagens resulting as by-products of metabolism within mitochondria (Brown et al., 1979; Lynch, 1997). MtDNA is often used for phylogenetic reconstruction between closely- and more distantly-related taxa, due to the relatively high rate of mutation. The regions used in this study were the NADH 2 subunit (ND2), the NADH 4 subunit (ND4) and cytochrome *b*.

Subspecies Classifications

Much discussion has occurred over the use of subspecies for classification, as definitions of what a subspecies "is" have often been inconsistent. The importance or significance of using a trinomial naming system has also come into question- of what use are subspecies? In early discussions of subspecies, researchers like Darwin suggested subspecies were groupings of individuals on their way to becoming fully-separated species (Mayr, 1982). Alternatively, subspecies were thought to be evidence that localized adaptations exist for specific habitat and climatic conditions within a larger biological species complex; what some might refer to as ecotypes.

A series of essays written by ornithology curators and researchers in the early 1980's elucidated concerns about subspecies descriptions. In almost all cases, uses for subspecies were supported. Subspecies classifications have become useful to describe geographic differences within sub-groupings of a species (Mayr, 1982; Parkes, 1982), and therefore related evolutionary patterns can often be inferred from morphological descriptions. Often a zone of intergradation is found between subspecies, where mixed phenotypes are observed. Barrowclough (1982) also supported the use of subspecies, particularly if the morphology or characters used to describe differences can be used in a predictive manner. Storer (1982) described a subspecies as a step in organization between populations and a species, with practical yet no theoretical use.

Currently subspecies can be important in describing localized population differences for both management and economic development reasons. Certain subspecies, for instance within *Thamnophis sirtalis,* have been given special status as endangered or threatened based upon subspecies classifications (Cover and Boyer, 1988).

T. s. infernalis, the San Francisco or California garter snake, has been listed as threatened due to over-collection for the pet trade, and habitat destruction within the area which it exists (Ernst and Ernst, 2003). The endangered Concho water snake, *Nerodia paucimacula,* has also been hypothesized as a subspecies of the Brazos River water snake, *Nerodia harteri*, which has caused difficulty in management of these snakes and land-development within the riverine areas these populations inhabit (Quammen, 1997; Ernst and Ernst, 2003).

Taxonomic History of *Thamnophis sirtalis sirtalis* and *T. s. pallidulus*

Thamnophis sirtalis is a species known for high phenotypic diversity across the range. Two of the twelve phenotypically-described subspecies live within the northeastern United States: *Thamnophis sirtalis sirtalis* and *T. s. pallidulus.* Most literature on *T. sirtalis* places the boundary between these two subspecies diagonally from southeastern New Hampshire to northwestern Vermont. *T. s. pallidulus* exists north and east of this region, and *T. s. sirtalis* exists south and west of this boundary (DeGraaf and Rudis, 1983; Conant and Collins, 1991; Rossman et al., 1996; Tennant, 2003).

Thamnophis sirtalis sirtalis was the first *T. sirtalis* individual described by Linneaus in 1758 (Fitch, 1980; Rossman et al., 1996). The Eastern garter snake, *T. s. sirtalis,* typically has dark brown or black coloration on the dorsal side, three yellow dorsal stripes, with greenish to yellow ventral scales (Ernst and Ernst, 2003). This eastern subspecies has one of the largest ranges of any *T. sirtalis* subspecies. *T. s. sirtalis* is found from the Atlantic coast west to Minnesota, as far south as Louisiana and Florida, and, in New England, as far north as southern New Hampshire and central Vermont. *T. s. sirtalis* remains one of the better-studied subspecies of *T. sirtalis* as many aspects of life-

history are well-studied. Aspects such as feeding ecology (Carpenter, 1952), hibernation tendencies (Costanzo, 1985), reproductive measures (Zehr, 1962), growth patterns (Burt, 1928), gene-flow and thermoregulation (King, 1988; King, 1993a; King, 2003), predator attacks (Placyk and Burghardt, 2005) and mating patterns (Blanchard, 1943) are all well-studied.

Thamnophis sirtalis pallidulus has remained one of the least-studied subspecies since its original description in 1899 by Allen (Allen, 1899). In this first description of individuals taken from Intervale, NH, Allen described a garter snake similar to "*T. sirtalis* proper". According to Allen, the Northern garter snake (later renamed the Maritime garter snake), *T. s. pallidula* (later renamed *T. s. pallidulus*), was olive colored, had a dorsal stripe "except at its inception, almost obsolete," and had interlinear spots of reddish scales with slim black edges and black interspaces. Allen described the ventral side as gray to white in young specimens, and in adults as light gray to light yellow. Allen thought this was a different taxon from Linnaeas' *Coluber sirtalis* (later renamed *T. s. sirtalis*), as Linneaus' sample of *T. sirtalis* showed three distinct blue-green stripes. Linnaeas would have seen specimens preserved in alcohol, which he received from Kalm who sampled in northern New York, and Southern Quebec. Alcohol preservation would have created "greenish-blue" stripes from yellow. In his description, Allen also gave reason to think the morphology of snakes captured in Intervale was widespread within eastern Canada and the extreme northeastern United States. Allen had captured specimens in Caribou, Maine, and had personal communication with a naturalist in Pictou, Nova Scotia, who had observed similar snakes. Allen also suggested that similar

color morphologies had been found further south into Massachussetts, perhaps suggesting hybridization (Allen, 1899).

Bleakney (1959) renewed the description of *Thamnophis s. pallidula* in 1959, with more samples taken from a broader range in eastern Canada. Bleakney agreed with Allen that a larger-scale pattern in morphology was seen in eastern Canada and northern New England with a brown to olive dorsal coloration, and two to three gray to white dorsal stripes. However, since Allen's article in *the Proceedings of the Boston Society of Natural History* in 1899, support for Allen's claims remained scant. Bleakney looked at museum specimens taken from various regions in Canada, and decided that there was substance to Allen's original claims. He noticed the olive coloration, and noticeable lack of dorsal stripe in many samples. When samples did possess dorsal stripes they were grey, not yellow as in other *T. sirtalis* specimens found further south and west.

Thamnophis s. pallidulus research remained limited until Rye (2000) studied *T. sirtalis* intergradation across Canada with a variety of methods: 364 bp cytochrome *b*, restriction fragment length polymorphism (RFLP) markers, ventral scale counts, and allozymes. Results from this study suggested that different clades of *T. sirtalis* snakes do exist within Eastern Canada and the United States. Rye showed that a "maritime" and an "eastern" clade of *T. sirtalis* garter snakes do exist based on cytochrome *b* sequence data. Furthermore, ventral scale counts did also agreed with the cytochrome *b* phylogenies, as did the allozyme electrophoresis results. Rye's results suggested that snakes belonging to the "maritime clade" would exist as far south as Rhode Island and as far west as central New York, where according to other field guides based on morphological patterns, these

areas are almost exclusively inhabited by the Eastern garter snake, *T. s. sirtalis*. Therefore questions on subspecies classifications still remain.

Much is also left unknown about *Thamnophis s. pallidulus* in terms of life history. Allen described this subspecies as living mostly in woods, and it was observed consuming amphibians, including wood frogs (*Rana sylvatica*), and American toads (*Bufo americanus) (*Allen, 1899*)*. Barnes et al. (2006) published a more comprehensive work on many life history traits of *T. s. pallidulus* from one population on Georges Island, Nova Scotia. Their studies showed three separate morphs of snakes on the island: striped, unstriped, and melanistic (Barnes et al., 2006). This study found smaller adult snakes in this location as compared to mainland Nova Scotia garter snakes. Reproducing females (N= 4) in Kejimkujik, Nova Scotia (Gregory and Larsen, 1993), had to achieve a minimum size of 46.4 cm snout-vent length (SVL), whereas on Georges Island, female snakes were reproductive at 35 cm SVL (Barnes et al., 2006). Only four out of 359 snakes captured exceeded 50 cm SVL. Also, SVL of neonate snakes was between 11 cm and 11.5 cm, which is smaller than previous reports of Nova Scotia neonates. Georges Island garter snakes ate almost exclusively red-backed salamanders (*Plethidon cinereus*) and earthworms found in this region. Beyond this study of Georges Island snakes no natural history is known of maritime garter snakes besides the original descriptions of the species by Allen. For more information on reproductive ecology of snakes morphologically classified as *T. s. pallidulus* see chapter 1 of this thesis.

North American Snake Phylogenetics and Phylogeography

A number of studies have investigated phylogeography of colubrid snakes closely related to *Thamnophis sirtalis*. Phylogeographic studies provide information on

relatedness of populations within a species, and the evolution of how such species may have diverged, and why. Many such studies focus on the relationship of morphological descriptions to a relatively neutral molecular marker, to see where similarities and differences exist in classifications. The results from these inquiries also have management and conservation implications, as species and subspecies have been identified by such techniques.

One phylogeographic study was completed on the North American rat snake (*Elaphe obsoleta*) (Burbrink et al., 2000). This study investigated the relatedness of the eight described subspecies in this widely-distributed reptile, based on complete cytochrome *b* sequence, and the control region of the mitochondrial genome. Burbrink et al. found 59 different haplotypes within 73 *E. obsoleta* specimens collected from across the eastern United States. Three distinct mtDNA clades were found: west of the Mississippi River, between the Mississippi and Appalachian range, and east of the Appalachian Mountains. Individuals within each morphological subspecies were distributed throughout the three clades, in no specific order, suggesting that the subspecies do not represent ancient individual lineages. The genetic data revealed that the subspecies within *E. obsoleta* were invalid. Burbrink et al. suggested that subspecies classifications based on a few morphological characteristics could not only be false, but could also be detrimental to understanding the true evolution of the species. However, if mitochondrial lineages do not match morphological patterns, other investigations into the causes or relationships of these morphologies still warrant investigation.

In a study of the California mountain kingsnake, seven geographical races were divided into three genetic clades based on mtDNA sequence data (Rodriguez- Robles et

al., 1999). Another study of snakes previously described as *Pituophis melanoleucus,* or the pine, bull, and gopher snakes examined 13 subspecies, and two other species from the same genus. The results of this continent-wide phylogeographic study suggested that separate species should have been described as different lineages of *Pituophis melanoleucus*: one in the eastern United States, and one in the western United States (Rodriguez- Robles and Jesus-Escobar, 2000). These show the importance of such phylogeographic studies of subspecies, as certain populations of species can be labeled as rare and given special status as endangered.

Phylogenies within *Thamnophis*

The genus *Thamnophis* is known for high speciation within North America, and sometimes finite niche partitioning among species (De Queiroz et al., 2002). The first large-scale phylogenetic study charted allozyme and genetic variability within 307 base pairs of the cytochrome *b* region to test relationships amongst 26 species within the genus *Thamnophis.* Results suggested that allozyme and mtDNA tree constructions gave different answers (De Queiroz and Lawson, 1994). A more recent study which sequenced three complete mitochondrial regions totaling 3039 bp included *Thamnophis* species and species from the genus *Regina* (the related taxa of crayfish snakes) suggested the group likely evolved from one invasion of species from Mexico (Alfaro and Arnold, 2001).

de Queiroz et al. (2002) showed with more sequence data (3809 bp) a more complete and robust phylogenetic tree of *Thamnophis* species. Data showed a plateau occurred in phylogeny resolution before the 3809 base-pair maximum. This study suggested that phylogenetic relationships might become clearer, if in fact more

individuals from a given population or species are sampled, instead of maximizing base-pair numbers, particularly if high differentiation within one taxon is known. For instance, within *T. sirtalis* based on the 307 bp in de Queiroz and Lawson (1994) a 6.6% difference was noted between two individuals.

Bronikowski and Arnold (2001) looked into subspecies classifications of *Thamnophis elegans* from fourteen different populations within the western United States and Canada. Based on the 307 bp of cytochrome *b* sequenced, this experiment showed 57 variable sites. This yielded 22 haplotypes, and 7.9- 12% divergence between *T. elegans* samples and out-group *T. sirtalis* individuals. Within *T. elegans,* 0.3- 7.7% divergence was noted. The three out-group *T. sirtalis* individuals from Illinois, Washington, and California showed 0.3- 0.6 % divergence between them. Overall, the results suggested that morphological characters did not match mtDNA clades and thus subspecies classifications were not consistent and therefore invalid.

Other studies have focused on the phylogenetics and phylogeography of *T. sirtalis* directly. Janzen et al. (2002) studied the relationships of five described subspecies in western North America (Janzen et al., 2002). A total of 2217 base pairs were sequenced from the cytochrome *b* (576 bp), ND4 (591 bp), and ND2 mitochondrial regions (1050 bp) (Janzen et al., 2002). From those, 273 variable characters were found, when compared across all taxa, including *T. sirtalis* out-group specimens from Illinois and New York, as well as an out-group specimen of *T. elegans*. Within western *T. sirtalis* populations, 55 variable characters were found over the 2217 base series for a maximum of 2.5% sequence divergence within the taxon. The 19 different populations were placed into three distinct clades: Northwest Coastal (near the California- Oregon border and west

of the Cascade Mountains), Intermountain (east of the boundary describing the Northwest clade and in between the Cascade Mountain range and the Rocky Mountains), and the California clade, which consisted of all specimens further south. The resulting phylogenetic trees had no distinct clades coinciding with subspecies classification, suggesting also that morphologically-described *T. sirtalis* subspecies classifications should be ignored in the Western United States. Therefore, Janzen et al. suggested that morphological differences must have been shaped more directly by local evolutionary properties than by a common ancestry (Janzen et al., 2002).

Most recently Placyk et al. (2006) investigated the ND2 lineages of *Thamnophis sirtalis* from populations surrounding the Great Lakes region of the northern Midwest United States. Snakes were collected from eastern Ontario, New York, Ohio, Pennsylvania, Indiana, Michigan, Wisconsin, and Illinois. All individuals within this study were morphologically identified as *T. s. sirtalis*. The complete 1101 bp ND2 region was sequenced from 148 individuals, yielding 36 unique haplotypes from 37 populations. Seventy-two of the 1101 sites were shown to be variable, yielding a maximum 6.5% divergence of sequences. These haplotypes were placed into three evolutionary clades: Upper Peninsula Michigan, Wisconsin and Illinois; Lower Michigan; Ohio, eastern Ontario, eastern Michigan, Pennsylvania, and New York. These clades suggested three separate pathways were taken by reinvading garter snakes after the Laurentide Ice Sheet retreat. One clade began in Indiana and moved east of the Lake Michigan, inhabiting the Lower Peninsula of Michigan and the Beaver Island Archipelago in Northern Lake Michigan. The second group began in Illinois, invaded Wisconsin, and continued into the Upper Peninsula of Michigan joining the other

invading front on the Beaver Island Archipelago. All populations surrounding Lake Erie were thought to have invaded from Ohio or eastern Michigan. Placyk et al. acknowledged the overall high genetic diversity observed in the specimens sampled in this region, particularly as all individuals existed within one putative subspecies. This study showed how increasing sample size within one population can increase observed diversity, as most of the previous phylogenetic work with *Thamnophis* snakes had sampled from only one or two individuals within a population (Placyk et al., 2006).

Phylogeographic Studies from the Northeastern United States in other Taxa

Despite the lack of in-depth phylogeographic research on snakes from the Northeast, one can make hypotheses about *Thamnophis sirtalis* recolonization and evolutionary relationships from studies conducted on other taxa sampled within the northeastern United States. Three mammal species have been investigated phylogeographically: *Tamias striatus,* the eastern chipmunk; *Peromyscus leucopus*, the white-footed mouse; and *Blarina brevicauda*, the northern short-tailed shrew. Partial cytochrome *b* sequences for the two mammalian species *Tamias striatus*, and *Peromyscus leucopus*, suggested that these species exist as two clades: a western clade, and an eastern clade, which roughly divided at Ohio and places further south. These results also suggested that during glaciated times, these species must have existed in multiple refuges, one in the east, and one from the west or perhaps south. Also, both species must have existed in one similar refuge for the eastern clade (Rowe et al., 2006). Evidence also suggested that within *Tamias striatus* some individuals may have tolerated the colder climates in regions which remained ice-free (as in the western portion of Wisconsin), and may have actually reinvaded south after receding of ice-sheets (Rowe et al., 2004).

The Northern short-tailed shrew, *Blarina brevicauda*, has been phylogeographically investigated from samples taken in the Northeast as far north as New Hampshire, south to central Tennessee, and west to Nebraska (Brant and Orti, 2003). After sequencing the cytochrome *b* region from 76 individuals across this range plus two out-group *Blarina carolinensis* individuals, 131 out of the 1131 base-pairs were found to be variable. Two distinct clades were discovered, which were split east and west of the Mississippi River. Mean divergence between eastern and western groups was 2.5%. After closer inspection, the east clade could be separated east and west of the Appalachian Mountains. Authors of this work attributed fragmented habitat and resulting evolutionary time as reasons for divergences, despite similar habitats in all three geographic locations.

Pseudacris crucifer, the spring peeper, is one amphibian which likely expanded in a similar manner to *Thamnophis sirtalis* after the glacial retreat. *P. crucifer* also has a high cold tolerance and it exists in wooded habitats. It is one of the first amphibians to emerge and breed once the spring thaw begins in northern regions. One study focused on a range-wide phylogeography of *P. crucifer* across the whole range east of the Mississippi, north to northern Quebec and Ontario, and south to the Gulf Coast and central Florida (Austin et al., 2002). After sequencing part of the cytochrome *b* and 16S ribosomal RNA region of the mitochondrial DNA from 40 populations, 4 clades were observed. One clade existed in two different groupings in the southeastern United States east of the Appalachians: one existed between the Appalachians and the Mississippi river; and another existed west of the Mississippi as far north as central Illinois. The fourth clade existed in the northeastern United State through south-central Ontario, and into

northern Wisconsin and Minnesota. This pattern suggested a recolonization from the northeast in the fourth clade in which individuals moved west through Ontario, and then south into Wisconsin and Minnesota. It appeared as though there was some secondary contact between this northeastern lineage, and the lineage which colonized between the Appalachians and the Mississippi in southwestern Ontario above Lake Erie (Austin et al., 2002). Overall divergences within this study ranged from 0.3% to 3.5% for the 16S unit, and 0.2 % to 3.8% in cytochrome *b*. Hybridization between *P. crucifer* and other hylid frogs was also suggested for divergent lineages within these mitochondrial regions. Results of this study also suggest that subspecies classifications of these frogs were invalid (Austin et al., 2002).

In a comparative study between *Pseudacris crucifer* and *Rana catesbeiana*, the bullfrog, differing results were suggested for recolonization patterns (Austin et al., 2004). Within the 42 study sites from which *R. catesbeiana* was sampled, only two lineages were observed, instead of the three to four lineages observed in *P. crucifer* which were sampled from 60 sites within the same range east of the Rocky Mountains. Lower genetic diversity was observed in *R. catesbeiana* (<2%) when compared to *P. crucifer* (< 6 %) (Austin et al., 2004). These results suggested that *P. crucifer* used more refugia during glaciated times than did *R. catesbeiana*. Moreover, since *R. catesbeiana* was found to have only two lineages- east and west of the Mississippi, the authors suggested that physiographic borders such as the Appalachian mountains might not be as limiting to this species when compared to *P. crucifer*, as *R. catesbeiana* has more generalist habitat tolerances (Austin et al., 2004).

Other evidence suggests multiple patterns of recolonization in the Northeast after glacier recession. Church et al. (2003) studied tiger salamanders, *Ambystoma tigrinum* from across the eastern United States. This species exists within much of the eastern United States, however in more patchy distribution than other species. Church et al. showed that this species existed in two refuges through the Pleistocene: one which recolonized the Northeast, and another which remained in isolation within the Appalachian mountains and has since stayed in that region. Based on maximum-likelihood phylogenies, one clade consisted of individuals from Florida, Alabama, Tennessee, Michigan, and the Gulf Coast of Georgia, suggesting gulf-Coast populations recolonized the northern Midwest (Church et al., 2003). A second clade consisted of individuals from western Georgia, South Carolina, North Carolina, Virginia, New York and New Jersey. The eastern clade showed numerous divergences, mostly by state-location, suggesting continued isolation within these populations since expansion.

In summation, it appears that within the eastern United States the Mississippi River and the Appalachian Mountains have stood as barriers to recolonization for many ground-dwelling vertebrate taxa since glaciation. Moreover, morphological subspecies classifications have almost consistently disagreed with molecular data, suggesting that morphological relationships within taxa need to be more closely examined to suggest evolutionary relationships. Templeton (1995) lays out three biological reasons for divergence of haplotypes across a landscape: restricted gene flow, past fragmentation, and range expansion. All three of these reasons can be inferred as driving forces for differentiation in most phylogeographic studies.

Research Goals, Questions, and Hypotheses

This study aimed to reconstruct a mtDNA phylogeny of *Thamnophis sirtalis* snakes captured within northern New England. Recent studies have investigated these relationships within the western United States, and the Great Lakes Region, however the Northeast has been left relatively unstudied (Janzen et al., 2002; Placyk et al., 2006). In constructing this phylogeny, I proposed to sample across the subspecies boundary within New Hampshire between *T. s. sirtalis* in the south, and *T. s. pallidulus* to the north, to test if there is molecular reasoning for this difference in taxonomic subspecies classification which has existed for over 100 years since Allen's description in 1899. Moreover, I aimed to find evidence of the recolonization history of this species since glacial recession to try and suggest patterns of recolonization for *T. sirtalis* in New England. This study might then be used as a model to suggest recolonization history for other ground-dwelling animals.

The main null hypothesis of this study was:

H_0 : There is no difference between the two morphological subspecies sampled in this study based on mtDNA sequence data.

To reject my null hypothesis all snakes from the northern populations should lie within a monophyletic clade, when compared to specimens sampled further south, and there should be at least 2% mean divergence between clades (approximately 40 of the 2217 base-pairs to be sequenced). Other studies have suggested that subspecies differences would be supported strongly if up to 5% divergence in sequences was noticed.

I predicted that all snakes within this study would have high similarity (low divergence) when compared to other samples taken within the Northeast (one set of sequences is available from Central New York). The evidence from Rye (2000) and Burbrink et al (2000) suggests that snakes sampled from these regions showed high sequence similarity (Burbrink et al., 2000; Rye, 2000). In addition, I predicted that I would find no significant differences between snakes from the two subspecies because much previous research suggests that morphological subspecies tend not to equal distinct evolutionary lineages (Burbrink et al., 2000; Bronikowski and Arnold, 2001; Austin et al., 2002; Church et al., 2003; Austin et al., 2004).

Lastly, as mountain ranges and other major physiographic have served as barrierss to reinvasion in other parts of the continent, I investigated if the White Mountains in central New Hampshire also served as a dividing line of evolutionary lineages. To suggest they have, divergences would need to be observed North and South of this region, and high similarity would have to be observed within populations when compared to other populations.

The second null hypothesis was therefore:

H_0: There will be no substantial mtDNA variation among the five populations sampled.

Methods

Tissue Collection and Digestion

I clipped portions of ventral scales from live *Thamnophis sirtalis* snakes to obtain tissue. In 2005, I obtained tissue from 5 Westborough MA snakes, 6 Durham NH snakes, 3 from Ossipee NH, 1 from Berlin NH, and 1 from Pittsburg, NH. In 2006, four snakes were sampled from each of the following sites: Westborough, MA; Durham, NH; Ossipee, NH; Pittsburg, NH. Sampling locations are shown in chapter I, Figure 1. The tissue samples were placed in 100% ethanol, and stored at -20 °C until DNA extraction procedures were implemented.

Samples were digested with 0.4 mg/ml Proteinase K (Promega) in 500µl samples of extraction buffer (10mM Tris (pH 8.0), 2mM EDTA (pH 8.0), 200mM NaCl, 1% SDS, 8mg/ml Dithiothreitol (DTT)) in 1.5 ml silica-gel filled microfuge tubes. Chemical digestions ran 1.5- 2.5 hours at 37 °C.

DNA Extraction

Following digestion, I employed standard phenol- chloroform extraction techniques, followed by ethanol precipitation. 400µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added to each sample tube. Tubes were vortexed, and centrifuged at 15,000 RPM for 5 minutes inside a chemical safety hood. After centrifugation, a 300 µl portion of Chloroform: Isoamyl alcohol (24:1) was dispensed into each tube. The tubes were again centrifuged at 15,000 RPM for 5 minutes, and then the supernatant of each tube was poured into a new 1.5ml microfuge tube. 700 µl samples of chilled 100% ethanol mixed with 35 µl of NaOAC were added to each tube. Tubes were then vortexed and were refrigerated at 4 °C for 45 minutes.

Following refrigeration, microfuge tubes containing DNA supernatant and chilled ETOH mixture were centrifuged for 15 minutes again at 15,000 RPM at room temperature, and all supernatant was discarded. The tubes containing pelleted DNA were then placed into a vacuum-centrifuge for 5-10 minutes to remove any remaining liquid. Dried pellets were resuspended in 400 µl of 0.1M TE buffer (pH 8.0) and stored at 4 °C until DNA amplification.

DNA Amplification

Primer sequences based on Janzen et al. (2002) are shown in Table 2.1. Each polymerase chain- reaction tube contained the following: 2 µl 10x PCR buffer, 1.6 µl 2.5 M magnesium chloride (MgCl2), 1.6 µl DNTp's, 0.5 µl 10mM of each of the two primers within the set, 12 µl of deionized H_20, and 0.25 µl Taq polymerase enzyme. After vortexing this reaction mixture, 2µl of each of the extracted DNA samples were placed into the tubes to create an approximate sample volume of 20µl.

Table 2.1 : Primer sets used in to sequence partial cytochrome B, ND4, and complete ND2 mitochondrial regions in *Thamnophis sirtalis* snakes.

Coding Region	Primer Name	Primer Set #	Sequence (5' to 3')
cytochrome B	H15544	1	AATGGGATTTTGTCAATGTCTGA
cytochrome B	LGLU	1	TGATCTGAAAAACCACCGTTGTA
ND4	DW1641	2	TGACTACCAAAAGCTCATGTAGAAGC
ND4	DW1642	2	TATTAGTAGGTGTTCTCG
ND2	CE2330	3	CTAATAAAGCTTTCGGGCCCATAC
ND2	H5051	3	TCGGTGCTATTTTTAGTGTTGCTA
ND2	CE2331	4	TTCTACTTAAGGCTTTGAAGGC
ND2	L4956	4	CTATTATGCGCCACCCTATCAAT

The PCR cycle consisted of the following heating regime for 30 cycles: 95 °C for 2 minutes 30 s, 52 °C for 30 seconds, followed by 72 °C for 5 minutes. Refrigeration at 4 °C followed the reaction tubes.

PCR products were electrophoresed at 95 volts on 1% agarose gels in 1% TAE buffer, for 20 to 30 minutes and then stained with ethidium bromide (EtBR) for observation under ultraviolet light.

DNA Purification

Mitochondrial DNA products were purified using the solid phase reversible immobilization technique (S.P.R.I .). I added 25 µl amounts of hybridization buffer (2.5M NaCl, 20% PEG 8000) and 5µl magnetic beads mixture (1:5 dilution with 0.5M EDTA) to each DNA product solution. Samples were then resuspended and transferred to a 96 well plate fitted for the magnetic bead separation, and incubated for 10 minutes at room temperature. After incubation, I placed the DNA samples on the magnetic separation plate for 3 minutes, before aspirating the supernatent, leaving a pellet of magnetic beads and DNA adhered to one side of the 96 wells. The bead pellets were then washed twice with 150µl 70% ethanol while still on the magnetic plate, and then allowed to air dry for 1 hour.

Post-drying, 25µl of 10mM Tris elution buffer were added to each well, and beads were resuspended unattached to the magnetic plate. After an additional 3 minutes of incubation, the 96 well plate was returned to the magnetic plate for particle separation. DNA remained in solution after this last separation, and was then pipetted off and placed into new 50µl tubes.

Sequencing Chemistry

In preparation for sequencing, purified DNA products were single-stranded amplified with dideoxynucleotides in the Sanger Dideoxy chain termination method. This method was employed for all samples collected in summer of 2005.

2μl of the purified DNA template was added to 4μl DYEnamic Terminator mix (Amersheim Biosciences), 1μl 10mM primer, and 3μl deionized water for a reaction mixture of 10μl. These reaction mixtures were then thermocycled at 95 °C for 20 seconds, 50 °C for 15 seconds, and 60 °C for 60 seconds. This sequence of temperature fluctuations was employed for 25 cycles.

To separate the DNA from the reaction solution I precipitated it with EtOH. I prepared a mixture of 40:1 95% ETOH and sodium acetate EDTA buffer, and pipetted 41μl into 1.5 ml centrifuge tubes. Then single-stranded DNA product was added one to each 1.5ml tube, and then another centrifugation occurred at 12,000 RPM for 15 minutes at room temperature. All excess liquid was poured off, and 250μl volumes of 70% ethanol were added to the centrifuge tubes; all tubes were once again centrifuged for five minutes at 15,000 RPM. Ethanol was then poured off and tubes were then vacuum-centrifuged until pellets were completely dry. These tubes were then stored at -20 °C until sequencing could occur.

Sequencing of mtDNA samples was done on an ABI 377 sequencer on a 4% acrylamide gel at the Sequencing facility at the Hubbard Center for Genome Studies, (HGCS) Gregg Hall, Univeristy of New Hampshire.

In 2006, a capillary sequencer was employed for DNA sequencing. In this case purified DNA products were submitted to the sequencing facility at HGCS, and single-

strand amplification and subsequent purification was completed by staff of the sequencing facility.

Phylogenetic Reconstruction

Sequences were analyzed with the Sequencher 4.5 program, or FinchTV for quality and edited by hand for errors. They were aligned with ClustalW associated with the Mega 3.1 software program. Phylogenetic reconstruction was created as a neighbor-joining method assuming a Kimura 2 parameter correction also in MEGA 3.1. Out-group taxa were *Thamnophis sirtalis* individuals sequenced by Janzen et al. (2002) from New York, Illinois, California, and one *T. elegans* specimen. Boot-strap values were acquired after 500 replicates were repeated.

Results

Two differences in the cytochrome *b* sequences were observed among the five populations sampled from New England, creating two different haplotypes based on this region (Table 2.2). This created maximum 2 base-pairs differences between the *T. sirtalis* cytochrome *b* sequence from New York published by Janzen et al. (2002), however 28 base-pairs were different between the New England snakes and one sample from Illinois for a total of 4.82 % difference (Table 2.2). Thirty-three base-pairs differed between *T. sirtalis* sampled from McCumber County, CA and the New England snakes. In addition 69 base pairs differed (11.98%) between New England snakes and *Thamnophis elegans* (Table 2.2). Figure 2.1 shows a neighbor-joining tree based on the partial cytochrome *b* region from *Thamnophis sirtalis* snakes from the five populations studied in 2005 and 2006. High boot-strap values support the phylogenetic tree branches showing differences between *T. elegans* from all *T. sirtalis* individuals. Figure 2.1 also shows a clade composed of midwestern and western *T. sirtalis* snakes, when compared against snakes from New York and further east (Figure 2.1). Divergence between snakes from New York and further east was low, which is supported by boot-strap values (60% or below) and by the high homogenization of populations within the clade after boot-strap repetitions (Figure 2.1).

Table 2.2. Pairwise differences in *Thamnophis sirtalis* mtDNA sequences.

Region	# base pairs sequenced	Variable sites within New England *Thamnophis sirtalis*	Variable sites with New York out-group *Thamnophis sirtalis* (Janzen et al. 2002)	Variable sites with Illinois out-group *Thamnophis sirtalis* (Janzen et al. 2002)	Variable sites with California out-group *Thamnophis sirtalis* (Janzen et al. 2002)	Variable sites with out-group *Thamnophis elegans* (Janzen et al. 2002)	Figure
Cytochrome *b*	576	1 0.17%	2 0.34%	28 4.82%	33 5.73%	69 11.98%	2.1
NADH 4	591	1 0.33%	2 0.33%	23 3.90%	26 4.40%	71 12.01%	2.2
NADH 2	1050	2 0.19%	4 0.38%	49 4.67%	52 4.95%	110 10.47%	2.3
Total	2217	4 0.18%	9 0.41%	103 4.65%	111 5.01%	252 11.62%	2.4

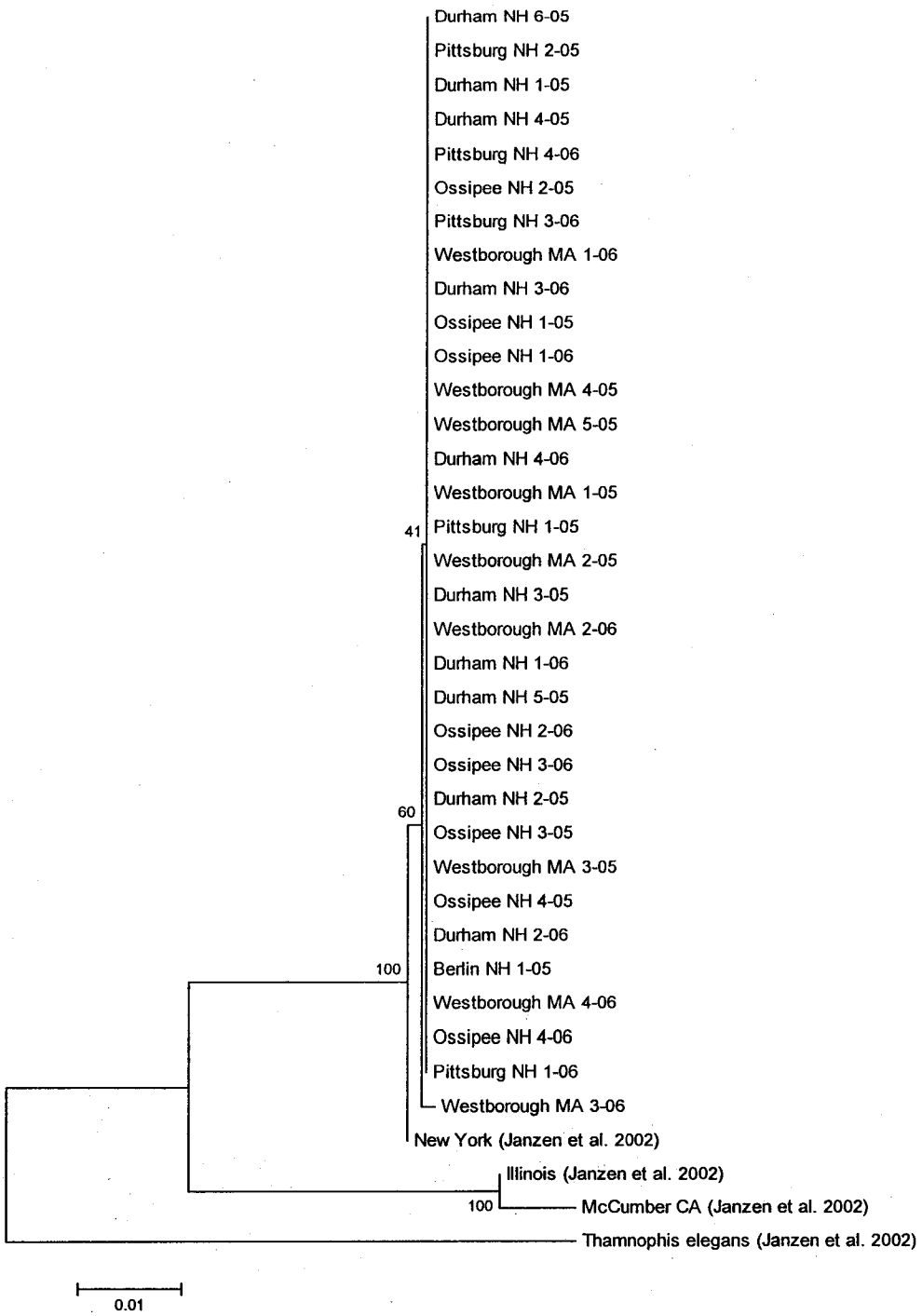


Figure 2.1. *Thamnophis sirtalis* cytochrome *b* phylogeny. This phylogenetic tree was created using a neighbor-joining method from 576 base-pairs of the *Thamnophis* mitochondrial genome. All individuals labled with a location are *Thamnophis sirtalis* garter snakes captured from New England.

Table 2.2 shows two differences exist among New England snakes in NADH 4 sequences, creating three different haplotypes found within the New England populations. Pair-wise comparisons between New England snakes and that of the Janzen et al. (2002) sequence from New York show one maximum difference (Table 2.2). Twenty-three base-pairs differed between the Illinois out-group *T. sirtalis* and the New England snakes for a 3.90% difference, and 26 sites differed from the California specimen and New England snakes for 4.40% divergence. Also 71 base-pairs differed between *Thamnophis elegans* and the New England *T. sirtalis* snakes for 12% difference (Table 2.2). Figure 2.2 shows a neighbor-joining tree based on the partial NADH 4 subunit of 591 base-pairs. Again, *T. elegans* and *T. sirtalis* existed upon different branches as well as did the Illinois and California specimen from the other *T. sirtalis* populations sampled further east (Figure 2.2). Within the eastern clade, a smaller difference is observed between five snakes from Pittsburg, NH and all other eastern snakes (one base-pair). In addition, two snakes from Westborough exist separately, however this is only based on one base-pair difference. Beyond this separation, again populations from the New England snakes were well-mixed within the eastern clade.

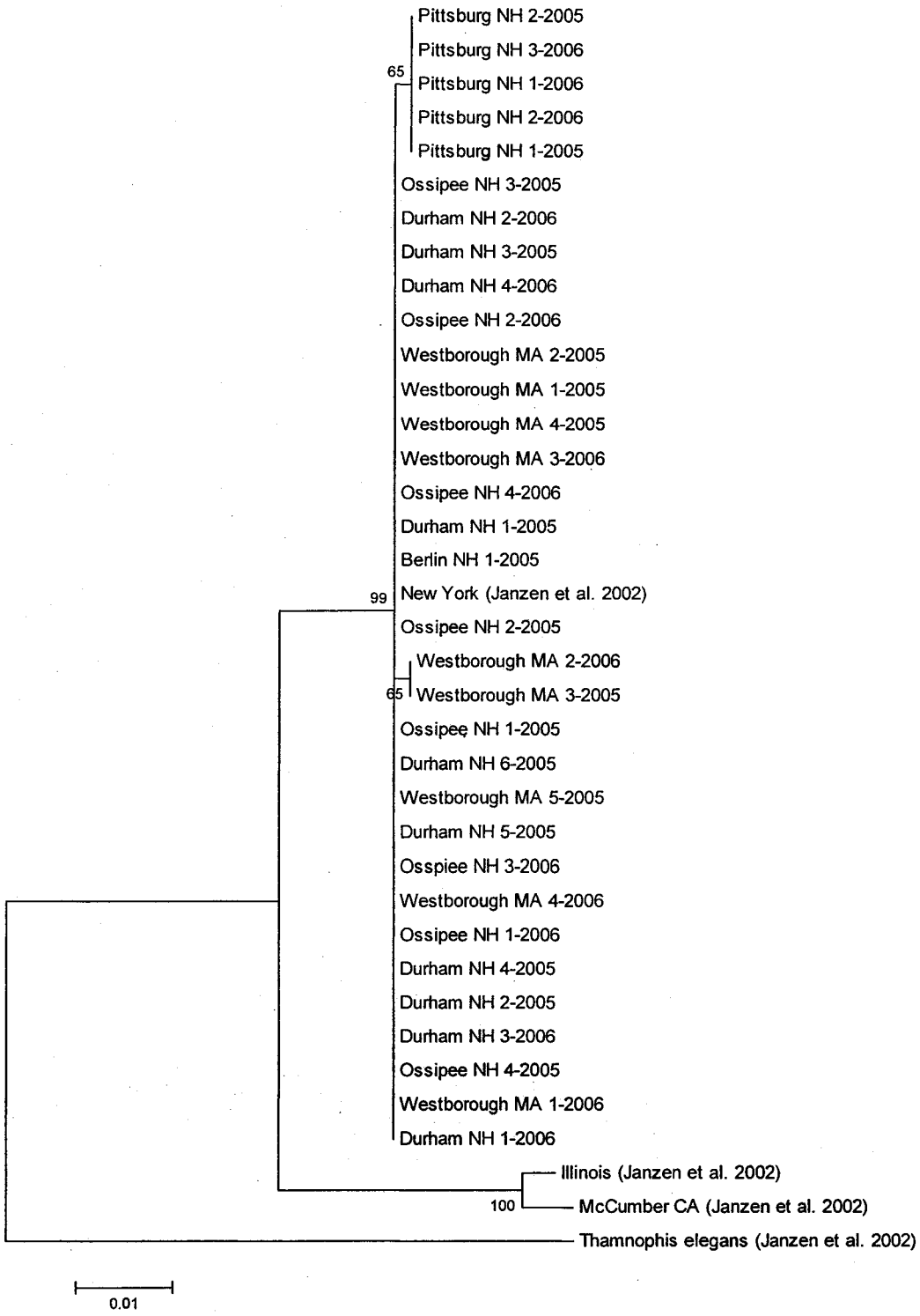


Figure 2.2. *Thamnophis sirtalis* NADH 4 phylogeny. This phylogenetic tree was created using a neighbor-joining method, and it is based upon 591 base-pairs of the *Thamnophis* mitochondrial genome. All individuals listed with a location and a year are *Thamnophis sirtalis* snakes captured from New England.

One-thousand-fifty base pairs were sequenced of the NADH 2 subunit and two differences were observed among populations in New England, creating three different haplotypes (Table 2.2). However, a maximum four sites differed between the New York out-group *T. sirtalis* and the New England snakes. Forty-nine sites differed between the Illinois *T. sirtalis* out-group, and the New England populations (Table 2.2). One-hundred-ten sites differed for 10.47 % difference between the *Thamnophis elegans* out-group and the *T. sirtalis* snakes sequenced from New England populations. These relationships are represented in Figure 2.3. Again, all snakes from New England are closely related with a smaller portion of snakes from the Northern Populations existing in a separate taxon by one base-pair. Illinois and California specimens are further related on a separate branch also.

Figure 2.3. *Thamnophis sirtalis* NADH 2 phylogeny. This phylogentic tree was created using the neighbor-joining method, and was based on 1050 base pairs of the *Thamnophis* mitochondrial genome.

Figure 2.4 shows a neighbor-joining tree created from all three regions sequenced in Figures 2.1-2.3. In total, four variable sites were observed among snakes sampled in New England, creating five haplotypes among New England populations (Table 2.2, Figure 2.4). Haplotype assignments are found in Table 2.3. Westborough snakes exist as one of three haplotypes; Durham snakes exist as one haplotype; Ossipee snakes exist as one of two haplotypes; the Berlin snake exists within one haplotype (only one snake was sampled); and Pittsburg snakes exist within one haplotype. Comparing New England mtDNA sequences to the sequence from New York reveals nine variable sites (Table 2.4). The New England clade compared to Illinois and California sequences shows 103 and 111 variable sites respectively for 4.65% and 5.01 % differences. Two hundred fifty two site differences were observed between New England snakes and *Thamnophis elegans* for an overall 11.62% divergence.

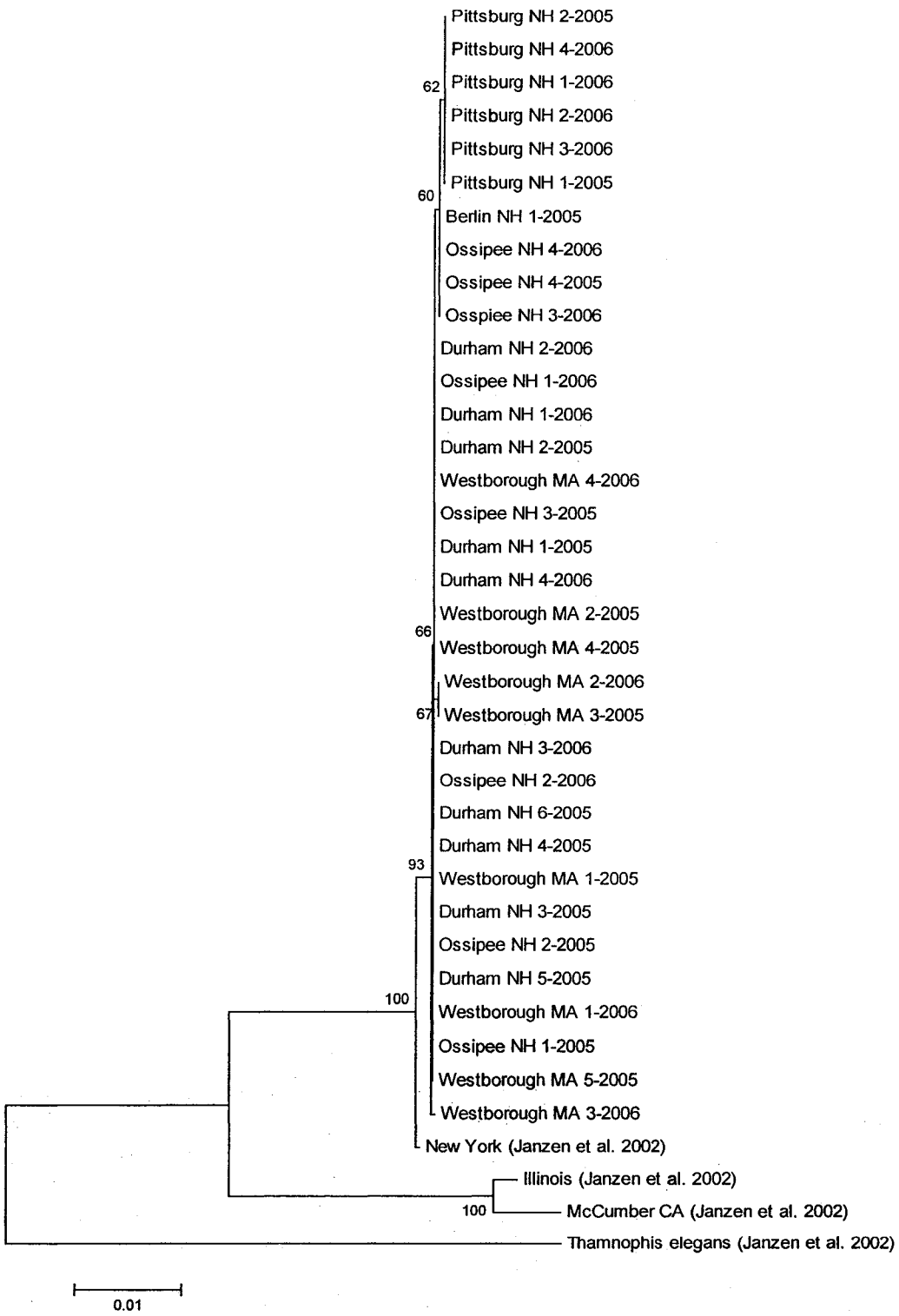


Figure 2.4. Concatenated *Thamnophis sirtalis* phylogeny. This phylogenetic tree was created by neighbor-joining method from 2217 base pairs of the *T. sirtalis* mitochondrial genome from the cytochrome *b*, ND4, and ND2 regions. The bootstrap values listed resulted after 500 replicates.

Table 2.3 Haplotype assignment table.

	# Individuals Sequenced	# Haplotype A	# Haplotype B	# Haplotype C	# Haplotype D	# Haplotype E
Westborough, MA	9	1	2	6	0	0
Durham, NH	10	0	0	10	0	0
Ossipee, NH	8	0	0	5	3	0
Berlin, NH	1	0	0	0	1	0
Pittsburg, NH	6	0	0	0	0	6

Discussion

This study set out to answer two general questions. The first question was whether or not the two morphologically described subspecies *Thamnophis sirtalis sirtalis* and *T. s. pallidulus* should be described as separate taxa based upon mtDNA differences. To reject the null hypothesis, snakes with the two distinct morphological patterns would have to exist within two different monophyletic clades, and there would need to be at least 2% divergence (approximately 40 characters out of 2217 total). Based upon the low divergence within populations of New England snakes (< 0.2%, Table 2.2) there is no molecular (mtDNA) justification to classify these snakes as separate subspecies. Therefore the initial null hypothesis should not be rejected. Snakes from different populations all existed within one monophyletic clade across all three mtDNA regions sampled (Figures 2.1-2.4).

Rye (2000) found a distinct "maritime" clade within New England *Thamnophis sirtalis* snakes based upon 348 base-pairs of the cytochrome *b* region, however the maritime clade boundaries were sampled as far west and south as central New York and Rhode Island. Snakes in this study were sampled from a smaller geographical region than Rye (2000). However because more sequenced base-pairs tend to resolve relationships further, as do more individuals from the same population (De Queiroz et al., 2002), I questioned if more distinct evolutionary clades would be noticed with larger-scale sequencing even in a smaller geographical sample (i.e. distinct clades for the morphological subspecies). The final results from this study support previous findings that no genetic difference between *T. sirtalis* snakes from New England exists. Molecular differences between *T. s. sirtalis* and *T. s. pallidulus* might still be found if

DNA samples were taken from the edges of their ranges, such as from populations further east. Much of the phylogeography of the Eastern garter snake *T. s. sirtalis* is well documented by Janzen et al. (2002), and particularly Placyk et al. (2006). A maximum 4% divergence is observed between snakes in my study and snakes from Illinois and further west, which does show some difference in evolutionary lineage between northeastern *T. sirtalis* populations and midwestern *T. sirtalis* populations (Table 2.2, Figures 2.1-2.4).

The second main question of this study inquired about the phylogeographic relationships between New England populations of *Thamnophis sirtalis* and populations sampled elsewhere without morphological characters being taken into account. In addition, I questioned if there was evidence that physiographic bodies, such as the White Mountains in Central New Hampshire, were barriers to recolonization after glaciation. Like the morphological comparisons, phylogenetic comparisons between New England populations showed very low divergence among populations (Table 2.2). These values are much lower than regional phylogeographic comparative studies conducted elsewhere (Placyk et al., 2006; Janzen et al., 2002). However, the total maximum physical distance between New England populations is approximately 300 km, whereas other studies in the Midwest and western United States were sampled over a much larger maximum distance.

The low divergence found within New England populations also suggests that the physiographic body of the White Mountain range in central New Hampshire was not a barrier to recolonization in *Thamnophis sirtalis*. In many other instances geographic bodies such as river systems or mountain ranges have served as hindrances for mtDNA flow, as was shown in the Northern short-tailed shrew (*Blarina brevicauda*) (Brant and

Orti, 2003), the North American rat snake (*Elaphe obsoleta*) (Burbrink et al., 2000), the Tiger salamander (*Ambystoma tigrinum*) (Church et al., 2003), and various species of frogs (Austin et al., 2004). However, these borders were much larger in size and scale (i.e. the Appalachian Mountain chain or the Mississippi River), than the White Mountains within New Hampshire.

Nonetheless, low divergence within the populations studied in this investigation can still lead to hypotheses about the evolution and recolonization of *Thamnophis sirtalis* within New England. Low divergence could be evidence that recolonization post-glaciation happened from one event, and it happened recently enough to not see many mutations within the mtDNA sequences from the five populations sampled in this study. Evidence to support this exists within phylogenetic studies of the spring peeper, *Pseudocris crucifer*, which showed one monophyletic clade from Pennslyvania north throughout New England and the Maritime provincess (Austin et al., 2002). Similar results were found by others in other taxa (Brant and Orti, 2003).

Future directions for this study could be many-fold. First, a finer-scale molecular marker could be used to judge more recent population gene-flow within these snake populations. One might be able to use microsatellite markers to find out if more recently physiographic boundaries such as the White Mountains in Central New Hampshire have served to block gene flow on a shorter time-scale.

Furthermore, it is still important to recognize that morphological differences between these scale-coloration patterns exist between snakes found north and east in New England compared to snakes observed south and west, even if mtDNA sequence data suggests these patterns are not deeply-rooted. One hypothesis to explain these

morphological patterns could be that there is a selective advantage in the northern and eastern regions of New England to express a brown or grey, more drab phenotype instead of the dark black and yellow-striped phenotype often described as *Thamnophis sirtalis sirtalis*. Perhaps this different phenotype described as *T. s. pallidulus* is more cryptic in the northern and eastern regions which are more dominated with pine (*Pinus sp.*), Eastern hemlock (*Tsuga canadensis*), fir (*Abies*) and spruce (*Picea sp.*) species. Previous researchers have used clay model snakes to test hypotheses of snake crypsis against avian predators (Bittner, 2003). Such a study could test morphological selection variation among snake populations from this investigation. Selection pressures would have to be very strong and similar across the whole range for this browner-grey color pattern to dominate as it does in the northern and eastern regions of New England and the Maritime provinces.

An alternative argument to the selection hypothesis is that this phenotype-difference developed early-on during recolonization, and spread as habitats became more tolerable. This hypothesis suggests genetic drift as the leading cause for the large-scale variation in morphological patterns between *Thamnophis s. pallidulus* and *T. s. sirtalis*. Selection could also have been acting in combination with drift as well.

It is also possible that the coloration differences might not have any selection component, and the differences are based upon some more simplistic small-scale genetic change resulting in a different phenotype. Some candidates for *Thamnophis sirtalis* scale color differences have been tested for the differences between melanistic individuals and normal striping. The McR1 gene was hypothesized as a candidate gene controlling differential pigment expression, but no large-scale patterns were noticed between

melanistic and normal-striping individuals (Rosenblum et al., 2004). Other investigations into other candidate genes controlling for differential gene expression could also elucidate other reasons for differences among *T. sirtalis* striping morphologies.

Lastly, I would like to comment on the implications this study as on the ecology and management of *Thamnophis sirtalis* within New England. This study has shown there is nothing distinct in terms of ancient lineages between the two morphological subspecies. In addition, neither of the "subspecies" appear to be limited in population size in any of the locations sampled throughout this study, or in areas further east and north (Barnes et al., 2006). Therefore, protecting these snakes currently with a threatened or endangered tag is unnecessary, but ample habitat should be maintained in all regions to support local populations and phenotypes.

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APPENDICES

APPENDIX A

UNIVERSITY of NEW HAMPSHIRE

June 30, 2005

Taylor, James
Zoology
Spaulding Life Science Center
Durham, NH 03824

IACUC #: 050202
Original Approval Date: 05/20/2005 **Modification Approval Date:** 06/30/2005
Review Level: B

Project: Investigations into molecular systematics and litter sizes of Thamnophis sirtalis in Northern New England

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the requested modification to the protocol for this study:

- *Per June 26, 2005 memo, request marking of snakes with PIT tags (chips) and replace blood draws with removal of single dorsal scale for source of DNA*

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Roger E. Wells, D.V.M.
Vice Chair

cc: File

Research Conduct and Compliance Services, Office of Sponsored Research, Service Building, 51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564

University of New Hampshire

May 3, 2006

Taylor, James
Zoology
Spaulding Life Science Center
Durham, NH 03824

IACUC #: 050202
Project: Investigations into molecular systematics and litter sizes of Thamnophis sirtalis in Northern New England
Category: B
Next Review Date: 5/20/2007

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your request for a time extension for this protocol. Approval is granted until the "Next Review Date" indicated above. You will be asked to submit a report with regard to the involvement of animals in this study before that date. If your study is still active, you may apply for extension of IACUC approval through this office.

The appropriate use and care of animals in your study is an ongoing process for which you hold primary responsibility. Changes in your protocol must be submitted to the IACUC for review and approval prior to their implementation.

Please Note:

1. All cage, pen, or other animal identification records must include your IACUC # listed above.

2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Roger Wells at 862-2726 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File

Research Conduct and Compliance Services, Office of Sponsored Research, Service Building, 51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564

Commonwealth of Massachusetts

Division of Fisheries & Wildlife

Wayne F. MacCallum, *Director*

Scientific Collection Permit

REPTILES AND AMPHIBIANS

VALID

2005

UNIVERSITY OF NEW HAMPSHIRE
WILLIAM KEAN
SPAULDING LIFE SCIENCES 119
DURHAM, NH 03824

DATE 4/1/2005
PERMIT#: 144.05SCRA

Subpermittee(s): JESSA WATTER, JAMES TAYLOR

is (are) hereby authorized, in accordance with the provisions of Section 4, Chapter 131 and 131A of the Massachusetts General Laws, to remove from the wild within the Commonwealth, subject to conditions set forth below, the following species and numbers:

MAY HAND CAPTURE EASTERN GARTER SNAKES STATEWIDE TAKING BLOOD AND TISSUE BIOPSY SAMPLES. MAY RETAIN UP TO 5 VOUCHERS FROM EACH LOCATION. ALL OTHERS MUST BE RELEASED AT THEIR SITE OF CAPTURE.

The following method(s) of taking is (are) hereby authorized:

HAND CAPTURE

Collection activites under this permit shall be restricted to the following locations, subject to the approval of private landowners

STATEWIDE

All specimens secured under this permit shall be donated to the following institutions:

RELEASE AT SITE OF CAPTURE. UP TO 5 VOUCHERS PER SITE MAY BE DEPOSITED WITH UNH

No specimen taken under the authority of this permit may be sold. No specimen may be transferred to another not duly licensed.

This permit of a copy thereof shall be carried at all times by the permittee and subpermittee(s) while engaged in the activities authorized herein.

This permit does not absolve the permittee from compliance in full with any and all other applicable federal, state and local requirements, including the acquisition of a federal endangered species permit if required.

Upon expiration of this permit, a complete report detailing all collection activities shall be filed with this office and must include a listing of all species taken, numbers of specimens, and the disposition of same.

This permit, unless sooner revoked for cause, shall expire on December 31 of the year of issue.

Wayne F. MacCallum

Wayne F. MacCallum, Director

Division of Fisheries & Wildlife
251 Causeway Street, Suite 400, Boston, MA 02114-2104
Phone: (617) 626-1590 Fax: (617) 626-1517 Web: www.masswildlife.org
An Agency of the Department of Fisheries, Wildlife & Environmental Law Enforcement

Commonwealth of Massachusetts

Division of Fisheries & Wildlife

Wayne F. MacCallum, *Director*

Scientific Collection Permit

REPTILES AND AMPHIBIANS

VALID

2006

UNIVERSITY OF NEW HAMPSHIRE
WILLIAM KEAN
SPAULDING LIFE SCIENCES 119
DURHAM, NH 03824

DATE 4/10/2006
PERMIT#: 116.06SCRA

Subpermitee(s) JESSA WATTER, JAMES TAYLOR

Is (are) hereby authorized, in accordance with the provisions of Section 4, Chapter 131 and 131A of the Massachusetts General Laws, to remove from the wild within the Commonwealth, subject to conditions set forth below, the following species and numbers:

MAY HAND CAPTURE EASTERN GARTER SNAKES STATEWIDE TAKING BLOOD AND TISSUE BIOPSY SAMPLES. MAY RETAIN UP TO 5 VOUCHERS FROM EACH LOCATION. ALL OTHERS MUST BE RELEASED AT THEIR SITE OF CAPTURE.

The following method(s) of taking is (are) hereby authorized:

HAND CAPTURE

Collection activites under this permit shall be restricted to the following locations, subject to the approval of private landowners

STATEWIDE

All specimens secured under this permit shall be donated to the following institutions:

RELEASE AT SITE OF CAPTURE. UP TO 5 VOUCHERS PER SITE MAY BE DEPOSITED WITH UNH

No specimen taken under the authority of this permit may be sold. No specimen may be transferred to another not duly licensed.

This permit of a copy thereof shall be carried at all times by the permittee and subpermittee(s) while engaged in the activities authorized herein.

This permit does not absolve the permittee from compliance in full with any and all other applicable federal, state and local requirements, including the acquisition of a federal endangered species permit if required.

Upon expiration of this permit, a complete report detailing all collection activities shall be filed with this office and must include a listing of all species taken, numbers of specimens, and the disposition of same.

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An Agency of the Department of Fisheries, Wildlife & Environmental Law Enforcement

New Hampshire
Fish and Game Department

11 Hazen Drive, Concord, NH 03301-6500
Headquarters: (603) 271-3421
Web site: www.wildlife.state.nh.us

TDD Access: Relay NH 1-800-735-2964
FAX (603) 271-1438
E-mail: info@wildlife.state.nh.us

Lee E. Perry
Executive Director

Daniel R. Lynch
Assistant Director

May 9, 2005

TO WHOM IT MAY CONCERN:

Under the authority contained in RSA 214:29, permission is hereby granted to: **William Kean, University of New Hampshire, Zoology Dept., Spaulding Life Sciences 119, Durham, NH 03824**

* to collect, temporarily possess the common garter snake. To quantify and summarize litter sizes for wild caught female and to compare litter sizes based upon latitude and study location to see if patterns exist in how female snakes invest in their offspring.

* to compare on a molecular basis the differences between the two-sub species, the maritime garter snake and the eastern garter snake.

* collection to take place from May – September 2005.

* to collect and house no more than 150 gravid female garter snakes.

* collection through the use of cover surveys and other general searching procedures.

* after collection, specimens will be housed at the University of New Hampshire in comfortable conditions until parturition. After birth, measurements of the offspring will be taken as well as adult females, and then return all but a few specimens to place of collection.

* a maximum of 6 adult snakes from each collection site will be euthanized for permanent collection.

* all studies will be conducted under the supervision of Dr. J. Taylor at the University of New Hampshire.

This permit or a copy shall be carried with the permittee while engaged in the above activity and shall be displayed to any Fish and Game Conservation Officer or employee upon request.

An annual report of turtles found and a summary of related activities shall be made to the Executive Director by January 31, 2006.

This permit shall expire on December 31, 2005 unless sooner revoked or rescinded.

Lee E. Perry
Executive Director

LEP/tc
cc: Law Enforcement Division
Wildlife Division\Nongame

Conserving New Hampshire's wildlife and their habitats since 1865.

New Hampshire
Fish and Game Department

11 Hazen Drive, Concord, NH 03301-6500
Headquarters: (603) 271-3421
Web site: www.wildlife.state.nh.us

TDD Access: Relay NH 1-800-735-2964
FAX (603) 271-1438
E-mail: info@wildlife.state.nh.us

Lee E. Perry
Executive Director

Daniel R. Lynch
Assistant Director

April 4, 2006

TO WHOM IT MAY CONCERN:

Under the authority contained in RSA 214:29, permission is hereby granted to: **William Kean, University of New Hampshire, Zoology Dept., Spaulding Life Sciences 119, Durham, NH 03824**

* to collect and temporarily possess no more than 50 garter snakes from each of four locations (Durham, Ossipeee, Berlin/Colebrook, and Pittsburg). To quantify and summarize litter sizes for wild caught female and to compare litter sizes based upon latitude and study location to see if patterns exist in how female snakes invest in their offspring.

* to compare on a molecular basis the differences between the two-sub species, the maritime garter snake and the eastern garter snake.

* collection to take place from May – September 2006.

* to collect and house no more than 200 gravid female garter snakes.

* collection through the use of cover surveys and other general searching procedures.

* after collection, specimens will be housed at the University of New Hampshire in comfortable conditions until parturition. After birth, measurements of the offspring will be taken as well as adult females, and then return all but a few specimens to place of collection.

* a maximum of 6 adult snakes from each collection site will be euthanized for permanent collection.

* all studies will be conducted under the supervision of Dr. J. Taylor at the University of New Hampshire.

This permit or a copy shall be carried with the permittee while engaged in the above activity and shall be displayed to any Fish and Game Conservation Officer or employee upon request.

An annual report of turtles found and a summary of related activities shall be made to the Executive Director by January 31, 2007.

This permit shall expire on December 31, 2006 unless sooner revoked or rescinded.

Lee E. Perry
Executive Director

LEP/tc
cc: Law Enforcement Division
Wildlife Division\Nongame

Conserving New Hampshire's wildlife and their habitats since 1865.

APPENDIX B

Date born	Date captured	Days in captivity	Female identification	Location	Adult female SVL (cm)
09/09/2005	06/23/2005	78	Mass 24	Westborough, MA	54.0
08/31/2005	06/23/2005	71	Mass 18	Westborough, MA	42.0
08/25/2005	06/23/2005	65	Mass 17	Westborough, MA	67.5
08/19/2005	06/23/2005	59	Mass 21	Westborough, MA	41.0
08/19/2005	06/09/2005	73	Mass 16	Westborough, MA	46.0
08/21/2005	06/03/2005	79	Mass 1	Westborough, MA	58.0
08/21/2005	07/01/2005	52	Mass 27	Westborough, MA	54.0
08/19/2005	07/01/2005	49	Mass 25	Westborough, MA	55.0
08/21/2005	07/01/2005	52	Mass 26	Westborough, MA	49.0
08/12/2005	06/23/2005	50	Mass 20	Westborough, MA	51.0
.	06/23/2005		Mass 19	Westborough, MA	
09/09/2005	06/05/2006	96	West Foss 4	Durham, NH	48.0
09/05/2005	06/05/2005	92	East Foss 3	Durham, NH	47.9
09/02/2005	06/05/2005	89	West Foss 3	Durham, NH	42.1
08/31/2005	06/21/2005	71	West Foss 12	Durham, NH	48.2
09/06/2005	06/05/2005	93	East Foss 2	Durham, NH	42.5
08/29/2005	06/13/2005	77	West Foss 6	Durham, NH	44.2
08/29/2005	06/05/2005	85	West Foss 2	Durham, NH	41.0
08/24/2005	06/05/2005	80	West Foss 1	Durham, NH	42.0
09/07/2005	06/13/2005	86	West Foss 7	Durham, NH	42.3
09/19/2005	07/21/2005	60	Ossipee 5	Ossipee, NH	44.1
09/12/2005	07/25/2005	49	Ossipee 9	Ossipee, NH	45.4
09/05/2005	07/21/2005	46	Ossipee 6	Ossipee, NH	48.2
09/14/2005	07/08/2005	68	Ct. Lake 1	Pittsburg, NH	56.3
07/29/2006	05/30/2006	60	Mass 4	Westborough, MA	63.8
08/13/2006	05/30/2006	75	Mass 5	Westborough, MA	42.8
08/07/2006	07/31/2006	7	Mass 41	Westborough, MA	46.0
08/07/2006	07/31/2006	7	mass 40	Westborough, MA	40.6
07/31/2006	06/05/2006	56	Mass10	Westborough, MA	43.5
07/31/2006	06/11/2006	50	Mass 14	Westborough, MA	42.2
07/31/2006	06/11/2006	50	Mass 12	Westborough, MA	47.4
07/30/2006	06/11/2006	49	Mass 13	Westborough, MA	46.4
07/28/2006	06/11/2006	47	Mass 19	Westborough, MA	40.3
07/27/2006	06/05/2006	52	Mass 6	Westborough, MA	55.0
07/27/2006	05/24/2006	64	Mass 1	Westborough, MA	57.2
07/25/2006	06/05/2006	50	Mass 11	Westborough, MA	51.2
07/29/2006	05/30/2006	60	Mass 2	Westborough, MA	50.4
07/28/2006	06/11/2006	48	Mass 15	Westborough, MA	52.1
07/27/2006	06/12/2006	45	Mass 25	Westborough, MA	54.1
07/25/2006	06/12/2006	43	Mass 26	Westborough, MA	49.2
07/29/2006	05/30/2006	60	Mass 3	Westborough, MA	53.8
07/27/2006	05/30/2006	58	Mass 9	Westborough, MA	55.2
08/05/2006	06/12/2006	54	Mass 34	Westborough, MA	50.5
08/06/2006	06/11/2006	56	Mass 17	Westborough, MA	61.2
07/31/2006	06/12/2006	49	Mass 28	Westborough, MA	50.2
08/03/2006	06/11/2006	53	Mass 22	Westborough, MA	44.1
08/03/2006	06/12/2006	54	Mass 29	Westborough, MA	40.2
08/02/2006	06/12/2006	53	Mass 24	Westborough, MA	36.3
08/02/2006	06/12/2006	53	Mass 32	Westborough, MA	39.2
08/01/2006	06/12/2006	52	Mass 30	Westborough, MA	49.0
08/05/2006	06/12/2006	56	Mass 35	Westborough, MA	44.9
08/05/2006	06/12/2006	56	Mass 33	Westborough, MA	41.0
.	05/30/2006		Mass 8 (died)	Westborough, MA	49.9
.	05/30/2006		Mass 7(died)	Westborough, MA	54.1
08/13/2006	06/14/2006	60	Ossipee 5	Ossipee, NH	52.1
08/08/2006	06/14/2006	55	Ossipee1 (died)	Ossipee, NH	51.8
08/17/2006	06/14/2006	64	Ossipee 6	Ossipee, NH	61.0
08/08/2006	06/14/2006	55	Ossipee 4	Ossipee, NH	48.7
08/12/2006	06/14/2006	59	Ossipee 2	Ossipee, NH	39.4
07/30/2006	05/29/2006	62	Durham 5	Durham, NH	49.7
08/02/2006	06/13/2006	50	Durham 13	Durham, NH	48.4
07/24/2006	05/29/2006	57	Durham 2	Durham, NH	40.1
07/26/2006	05/29/2006	58	Durham 9	Durham, NH	47.2
07/22/2006	05/29/2006	54	Durham 8	Durham, NH	43.2
08/07/2006	06/13/2006	55	Durham 15	Durham, NH	45.4
08/06/2006	06/27/2006	40	Durham 21	Durham, NH	46.5
08/03/2006	05/29/2006	66	Durham 7	Durham, NH	45.3
07/29/2006	05/29/2006	61	Durham 3	Durham, NH	40.0
08/01/2006	05/29/2006	64	Durham 4	Durham, NH	42.6
08/01/2006	06/06/2006	56	Durham 11	Durham, NH	45.1
08/06/2006	05/29/2006	69	Durham 6	Durham, NH	45.1
08/03/2006	05/29/2006	66	Durham 10	Durham, NH	43.2
08/14/2006	06/27/2006	48	Durham 19	Durham, NH	39.2

08/04/2006	06/23/2006	38	Durham 18	Durham, NH	45.1
08/07/2006	05/29/2006	70	Durham 1	Durham, NH	50.1
08/13/2006	08/09/2006	4	Durham 50	Durham, NH	44.7
08/06/2006	06/27/2006	40	Durham 20	Durham, NH	49.3
08/21/2006	08/09/2006	12	Durham 51	Durham, NH	46.2
08/21/2006	07/25/2006	27	Pittsburg 18	Pittsburg, NH	55.0
08/20/2006	07/06/2006	45	Pittsburg 5	Pittsburg, NH	44.5
08/22/2006	08/04/2006	18	Pittsburg 22	Pittsburg, NH	52.2
08/19/2006	07/06/2006	44	Pittsburg 8	Pittsburg, NH	55.9
08/18/2006	07/25/2006	24	Pittsburg 20	Pittsburg, NH	55.3
08/15/2006	07/21/2006	25	Pittsburg 11	Pittsburg, NH	46.4
08/16/2006	08/04/2006	12	Pittsburg 25	Pittsburg, NH	58.5
08/14/2006	07/25/2006	20	Pittsburg 19	Pittsburg, NH	49.1
08/13/2006	06/21/2006	53	Pittsburg 1	Pittsburg, NH	45.4
08/13/2006	07/19/2006	25	Pittsburg 10	Pittsburg, NH	44.6
08/14/2006	06/21/2006	54	Pittsburg 2	Pittsburg, NH	46.7
08/29/2006	07/21/2006	39	Pittsburg 14	Pittsburg, NH	56.7
08/25/2006	07/06/2006	50	Pittsburg 6	Pittsburg, NH	53.2
08/29/2006	08/21/2006	8	Pittsburg 27	Pittsburg, NH	44.1
08/26/2006	07/21/2006	36	Pittsburg 13	Pittsburg, NH	56.4
08/26/2006	08/04/2006	22	Pittsburg 24	Pittsburg, NH	52.2
08/25/2006	08/04/2006	21	Pittsburg 23	Pittsburg, NH	61.1
08/26/2006	07/25/2006	32	Pittsburg 21	Pittsburg, NH	59.6
08/26/2006	08/04/2006	22	Pittsburg 26	Pittsburg, NH	51.2
08/23/2006	07/25/2006	29	Pittsburg 16	Pittsburg, NH	47.6
08/22/2006	07/21/2006	32	Pittsburg 12	Pittsburg, NH	46.1

Adult female prepartum weight (g)	Adult female postpartum weight (g)	Offspring mean SVL (L+ S) (cm)	Offspring mean SVL (L) (cm)
95.5	60.000	12.300	
43.0	28.000	12.600	12.650
183.0	124.000	13.300	13.320
42.0	30.000	11.900	11.440
74.0	40.000	11.200	11.340
132.0	92.000	11.800	12.090
94.0	61.000	12.100	11.780
120.0	67.000	12.200	12.020
64.0	39.000	13.300	12.560
85.0	56.000	12.300	12.520
60.0	43.000	12.100	12.230
74.0	42.500	11.700	11.600
42.0	27.000	10.500	10.700
66.5	45.000	11.700	12.070
53.0	27.000	11.800	11.570
52.0	36.000	11.300	11.700
48.0	30.000	12.800	12.280
60.0	29.000	12.200	12.270
53.5	29.000	11.000	11.060
45.5	34.000	13.400	13.370
66.0	32.400	12.100	12.480
67.5	45.000	12.300	12.020
128.0	60.100	13.200	13.280
152.6	57.500	14.400	14.670
51.5	28.100		
79.2	50.000	12.410	12.410
42.7	28.300	11.200	11.200
65.1	33.800	12.070	12.070
60.0	28.700	11.620	11.610
81.6	41.000	12.690	12.690
69.0	30.200	12.130	12.130
42.0	18.900	10.750	10.690
97.1	49.500	12.150	12.150
140.0	60.000	12.740	12.740
84.0	44.600	11.660	11.650
95.8	46.000	13.290	13.280
95.0	44.100	11.890	11.890
98.0	51.200	12.180	12.210
68.2	44.600	10.880	10.880
104.3	46.800	12.800	12.800
123.2	67.900	13.030	13.020
75.3	37.300	13.120	13.120
120.0	69.500	13.480	13.480
90.0	37.800	12.500	12.530
65.1	25.600	11.980	11.980
48.0	27.700	12.100	12.100
49.0	18.800	11.700	11.700
46.7	35.300	11.820	11.820
70.0	33.200	11.740	11.910
61.8	27.700	13.380	13.380
43.1	31.800	13.000	13.000
80.2			
99.7			
65.6	41.400	12.420	
68.7			
116.2	64.200	12.220	12.220
52.5	30.900	11.350	11.350
40.0	17.200	11.960	12.010
86.9	45.000	13.170	13.240
83.9	40.100	12.630	12.850
37.2	19.100	11.640	11.640
61.5	45.400	12.410	12.420
46.4	22.200	12.710	12.710
62.6	36.700	11.810	11.780
72.8	37.900	12.970	12.970
77.5	37.200	12.090	12.090
42.1	21.600	12.610	12.610
42.1	24.200	12.930	12.930
65.0	33.200	12.090	12.090
57.2	28.500	12.720	12.720
58.7	26.900	11.990	11.990
40.7	24.400	11.600	11.600

66.2	29.400	12.750	12.750
94.1	53.800	13.850	13.730
58.6	31.500	13.200	13.200
84.5	42.500	14.420	14.420
64.8	40.600	13.570	13.200
100.5	53.800	14.180	14.180
63.7	37.400	12.450	12.450
91.8	54.800	14.480	14.480
102.8	54.500	14.950	14.950
102.9	51.400	14.560	14.560
63.2	48.900	13.480	13.480
157.4	74.600	13.930	13.930
82.9	50.700	12.720	12.810
47.5	22.500	13.040	13.210
49.5	26.800	13.060	13.050
65.7	34.700	14.220	14.220
105.0	56.800	14.500	14.300
94.6	48.000	13.500	13.500
52.7	32.600	12.510	12.510
102.6	64.400	14.420	14.410
90.1	45.800	13.420	13.430
173.4	87.500	13.390	13.390
106.9	70.800	14.700	14.700
97.4	46.500	14.030	14.020
67.9	38.500	13.270	13.530
59.5	37.000	13.660	13.700

Offspring mean weight (L+S) (g)	Offspring mean weight (L) (g)	Clutch mass (g)	Relative clutch mass	Clutch size	Year
1.280		26.850	0.448	23	2005
1.230	1.102	9.700	0.346	8	2005
1.350	1.292	26.080	0.210	24	2005
0.980	1.006	7.120	0.237	7	2005
1.070	1.093	23.750	0.594	20	2005
1.200	1.195	27.240	0.296	23	2005
1.180	1.138	18.370	0.301	16	2005
1.290	1.198	29.520	0.441	25	2005
1.400	1.258	13.090	0.336	12	2005
1.260	1.277	17.610	0.314	16	2005
				23	2005
1.380	1.297	11.300	0.263	9	2005
1.200	1.140	19.480	0.458	17	2005
0.870	0.952	7.640	0.283	9	2005
1.280	1.304	15.840	0.352	12	2005
1.270	1.275	16.580	0.614	13	2005
0.990	1.034	13.760	0.382	13	2005
1.230	1.200	14.380	0.479	12	2005
1.290	1.282	17.950	0.619	14	2005
0.920	0.940	13.410	0.462	14	2005
1.530	1.507	7.630	0.224	5	2005
1.280	1.257	20.110	0.621	16	2005
1.370	1.245	14.160	0.315	12	2005
1.740	1.675	41.860	0.697	25	2005
1.710	1.433	34.190	0.595	20	2006
0.762		12.950	0.461	17	2006
1.248	1.248	16.230	0.325	13	2006
0.757	0.840	4.540	0.160	6	2006
1.178	1.174	16.440	0.486	14	2006
0.909	0.909	10.910	0.380	12	2006
1.280	1.280	21.760	0.531	17	2006
1.133	1.133	23.790	0.788	21	2006
0.899	0.898	12.590	0.666	14	2006
1.260	1.260	27.730	0.560	22	2006
1.380	1.380	45.600	0.760	33	2006
0.952	0.948	18.080	0.405	19	2006
1.424	1.424	27.050	0.588	19	2006
1.163	1.163	23.260	0.527	20	2006
1.082	1.096	21.630	0.422	20	2006
0.885	0.885	16.820	0.377	19	2006
1.316	1.316	30.260	0.647	23	2006
1.383	1.385	38.730	0.570	28	2006
1.383	1.214	26.710	0.716	22	2006
1.431	1.431	30.060	0.433	21	2006
1.180	1.196	29.510	0.781	25	2006
1.114	1.114	17.830	0.696	16	2006
1.142	1.142	11.420	0.412	10	2006
1.057	1.057	11.480	0.611	11	2006
1.091	1.091	10.910	0.309	10	2006
1.052	1.106	22.100	0.666	21	2006
1.535	1.535	12.280	0.443	8	2006
1.340	1.340	8.040	0.253	6	2006
				31	2006
				11	2006
1.037		11.410	0.276	11	2006
				20	2006
1.178	1.177	35.330	0.550	30	2006
0.995	0.994	12.930	0.418	13	2006
1.072	1.069	11.790	0.685	11	2006
1.332	1.336	22.640	0.503	17	2006
1.338	1.387	21.400	0.534	16	2006
1.070	1.070	10.700	0.560	10	2006
1.271	1.270	13.980	0.308	11	2006
1.181	1.180	15.350	0.691	13	2006
0.949	0.931	17.080	0.465	18	2006
1.410	1.410	23.970	0.632	17	2006
1.061	1.090	21.220	0.570	20	2006
1.102	1.100	11.020	0.510	10	2006
1.263	1.263	11.370	0.470	9	2006
1.026	1.023	14.370	0.433	14	2006
1.272	1.272	17.820	0.625	14	2006
1.023	1.023	16.370	0.609	16	2006
0.864	0.800	6.910	0.283	8	2006

1.141	1.143	17.110	0.582	15	2006
1.560	1.456	21.840	0.406	14	2006
1.473	1.472	10.310	0.327	7	2006
1.610	1.618	27.520	0.648	17	2006
1.008	0.840	12.090	0.298	12	2006
1.678	1.678	28.530	0.530	17	2006
1.207	1.207	18.110	0.484	15	2006
1.966	1.966	27.530	0.502	14	2006
1.859	1.859	27.890	0.512	15	2006
1.789	1.789	34.000	0.661	19	2006
1.401	1.415	18.220	0.373	13	2006
1.795	1.795	53.850	0.722	30	2006
1.338	1.375	21.400	0.422	16	2006
1.192	1.234	10.730	0.477	9	2006
1.309	1.308	14.400	0.537	11	2006
1.871	1.871	18.710	0.539	10	2006
1.403	1.500	22.450	0.395	16	2006
1.629	1.629	29.320	0.611	18	2006
1.383	1.382	11.060	0.339	8	2006
1.675	1.674	18.420	0.286	11	2006
1.323	1.323	25.140	0.549	19	2006
1.440	1.440	50.410	0.576	35	2006
1.676	1.676	23.470	0.331	14	2006
1.606	1.606	25.700	0.553	16	2006
1.315	1.360	15.780	0.410	12	2006
1.326	1.346	15.910	0.430	12	2006